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Editorial

Academic publishing has undergone a revolution over the past decade. The time has come to decide whether the *Journal and Proceedings* will follow the revolution, or remain a niche publication only known to a few. The J&P has been going for over 150 years now. It hasn't remained entirely a print-only journal. We now publish online, through the RSNSW website, well in advance of the print edition. Past issues are available there electronically, as far back as the beginning of the 3rd millennium.

This doesn't provide for indexing, however. Papers will not be picked up by search engines except in special cases, and so scholars are unlikely to come across the journal when undertaking research. For the past two years the J&P has been indexed through Informit, which means that new articles can be picked up in library searches. Informit is, though, a subscription service, so access is not available to all. Fortunately all Australian universities and government agencies subscribe, making the J&P freely available to a wide audience within Australia.

All past issues of the J&P are also electronically available via the Biodiversity Heritage Library in the USA, who very kindly scanned every issue, going back to the first in 1862. This is an invaluable resource. Moreover, through optical character recognition, text versions of all volumes are available for searching. However, it still requires considerable effort to find material that might be of interest.

These advances, useful as they are, do not bring us into the era of modern publishing. If the Journal is to regain prominence as a publication of standing, it needs to do more.

We would need to move to a new platform for publication. There are two principal routes in front of us. The first is through a commercial publisher. They handle all the administrative details, and make the Journal available through their own platforms. Academic libraries subscribe to these, providing a world-wide audience. There is no cost to the Society, indeed it is possible for Journal to make money via these subscriptions. Sound too good to be true? Well yes it is, for the J&P has far too small a subscriber list to be financially worthwhile for a publisher. There is also the issue, strongly debated in academic circles, of the high profit margins being made by publishers, when the real work is being done by the Society and the authors.

The other alternative is through Open Access publishing. Here the RSNSW would pay for every article published, but this ensures that they remain freely accessible to all. Editorial and a variety of production services come with Open Access publishing, also necessary for improving the way publication is managed and distributed. Open Access is the altruistic method of publication, but will involve a cost to the Society. Not that our current method of publication is cost free either. The Society will need to decide on a way forward for our Journal to remain relevant 150 years hence.

Michael Burton
Hon. Secretary (Editorial)
30 June, 2015

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Forty Years of Photovoltaic Research at UNSW

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Abstract

The UNSW photovoltaic group started over 40 years ago in 1974, when the author was appointed as a young academic at UNSW. Within 10 years, the group had established an international reputation, achieving its first world record in the form of a silicon solar cell with independently confirmed 18% efficiency in 1983. This was a significant improvement upon efficiency levels initially established at Bell Laboratories and then further enhanced by the Communications Satellite Corporation (COMSAT, a company established specifically to develop US space communications technology). This record was the first of 18 successive internationally certified UNSW increments that took efficiency first beyond the 20% level, regarded us the "four-minute mile" of the field, eventually to 25%. The PERC (Passivated Emitter and Rear Cell) technology used to achieve the 25% result is now finding its way into commercial production as manufacturers follow the trail to higher efficiency earlier blazed in the laboratory. The PERC cell is expected to become the commercially dominant technology within the next 5 years as manufacturers inch towards 25% cell conversion efficiency in production.

Introduction

The origins of photovoltaics usually are traced back to 1839 when 19-year old Edmond Becquerel, working in his father's laboratory in Paris, measured electrical output from the electrochemical apparatus of Fig. 1a (Becquerel, 1839). It took nearly 40 years until Adams and Day demonstrated the effect in solid-state material. Working with selenium specimens originally prepared in 1873 for photoconductivity experiments by Willoughby Smith, they wondered whether it would be possible *"to start a current in the selenium merely by the action of light"* (Adams and Day, 1877). This proved to be the case although, with the required background theory still decades off, it was attributed to light-induced selenium crystallisation. From their publication, the author prepared the sketch of Fig. 1b. A colleague, John Perlin, recently tracked down these specimens in London, publishing a photograph similar to the sketch (Perlin, 2013).

The first photovoltaic visionary, Charles Fritts of New York (Fritts, 1883), made a large number of thin-film solar cells with the structure sketched in Fig. 2, sufficient to install an approximately 3-m^2 rooftop system in New York. An 1884 photograph of this system has also been published by John Perlin (Perlin, 2013). Although this was still before reticulated power supply was common, Fritts foresaw the possibilities that only now are becoming a reality, noting that *"the current, if not wanted immediately, can either be "stored" where produced, in storage batteries … or transmitted … to a distance, and there used, or stored …"*. Despite this enthusiasm, subsequent progress was slow, probably due to the on-going lack of a good theoretical explanation for the observed

effects. Selenium remained a common photovoltaic material up until the 1960s, due to the good match of its response to that of the human eye.

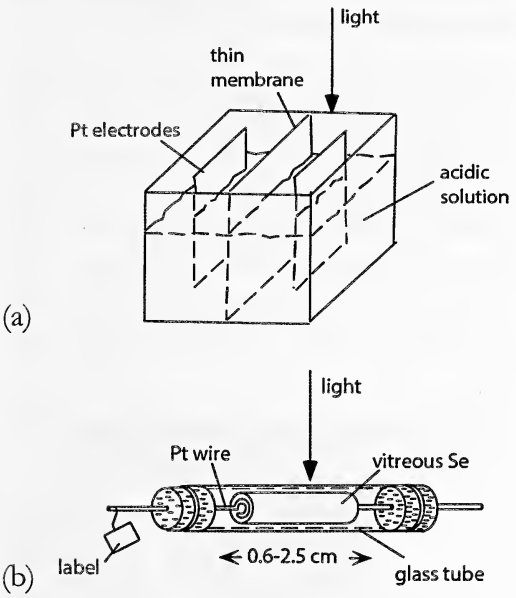

Figure 1: (a) Diagram of Becquerel's apparatus (1839); (b) Samples used by Adams and Day in 1876 for the investigation of the photoelectric effects in selenium.

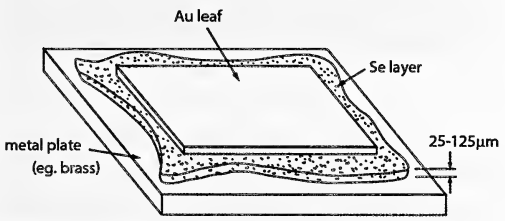

Figure 2: Thin-film selenium cell demonstrated by Fritts in 1883.

Einstein's explanation of the photoelectric effect during his "miraculous year" (Einstein, 1905) led ultimately to quantum theory and to Alan Wilson's subsequent explanation of semiconductor properties based on this theory (Wilson, 1931). Stimulated by growing interest in cuprous oxide devices (Grondahl, 1933), Walter Schottky then explained the photovoltaic effect as an interfacial effect caused by a barrier at the semiconductor surface (Schottky, 1938).

Schottky's work was crucial to the interpretation of a chance observation by Russell Ohl at Bell Laboratories in 1941 when he observed a photovoltage in slowly crystallised polycrystalline silicon specimens (Ohl, 1941). Pondering over this observation, the Bell Laboratories team correctly identified the effect as due to a barrier at the interface between silicon regions with different properties, *"like a Schottky barrier"*, with the material either side having properties described as positive (p-type) and negative (n-type).

Photovoltaics consequently was pivotal in the discovery of the p-n junction, the device that underpinned the subsequent microelectronics revolution. William Shockley published p-n junction theory in its full detail in 1949 (Shockley, 1949). This and associated technology development led to the reporting of the first efficient silicon solar cell in 1954 (Chapin et al., 1954). The associated press release made a big impact, with the results making it to the front page of the *New York Times* on April 24, 1954 (along with an update on the Petrov affair). This all seemed like ancient history to me when I made my first photovoltaic cell in 1971, but even Schottky's barrier work was closer to that date than is the present.

Silicon Cell Development

After demonstrating the first efficient silicon cell, Bell Laboratories and associated spin-off companies continued cell development, with application in space the main driver. Energy conversion efficiency improved rapidly, but stabilised at about 14% (under terrestrial sunlight) in the early 1960s. A decade later, COMSAT applied several of the substantial developments that had occurred in

microelectronics over the intervening years to improving cell technology (Lindmayer and Alison, 1973), with efficiency close to 17% by present standards established in 1974 (Haynos et al., 1974), the same year the author joined UNSW as a young academic.

The cell structure establishing this new performance level is shown in Fig. 4. This has served as the benchmark for subsequent work, since a relatively simple way of making a lower cost version was reported at about the same time, using a screen-printing approach to form the metal contacts (Ralph, 1975). By the early 1980s, this approach had become the industry standard, with this position only now being challenged by technology incorporating subsequent UNSW improvements in the form of PERC technology.

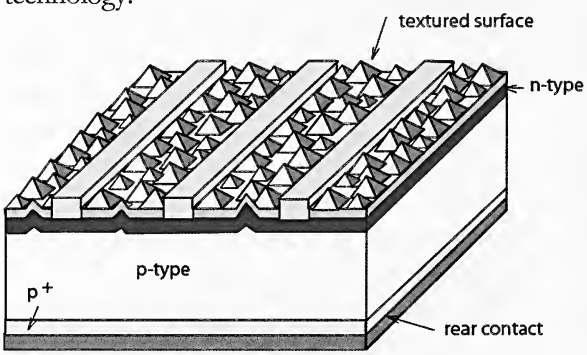

Figure 3: "Black cell" developed in COMSAT in 1974 (Haynos et al., 1974), essentially the same technology dominating the commercial production of terrestrial cells over a 30-year period from the early 1980s until recently.

The author's PhD thesis in Canada had investigated the properties of the Metal-Oxide-Semiconductor (MOS) structure when the oxide became sufficiently thin that electrons could pass through it by quantum mechanical tunnelling. One property identified was that these thin-oxide MOS devices could be designed to give properties identical to those of the ideal semiconductor p-n junctions that Shockley had earlier analysed. However, being free of some of the limitations of actual p-n junctions, they demonstrated superior properties in some applications (Green et al., 1974).

Working with my first PhD student, Bruce Godfrey, we reported our first notable result with this structure in 1976, demonstrating a 618 mV open-circuit voltage (Voc) at solar current densities (Green and Godfrey, 1976), one of the higher values for silicon at this time. Around this period, NASA-Lewis initiated a program to improve silicon space cell efficiency by improving Voc (Brandhorst, 1976). This started a competition between UNSW and several US groups being funded by NASA to achieve this outcome.

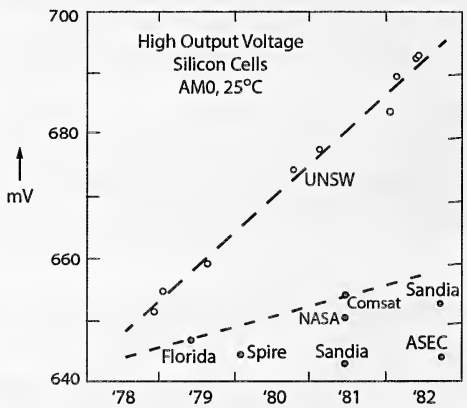

Figure 4: History of silicon cell Voc improvement showing the lead established at UNSW using surface oxide passivation.

We did well, as indicated in Fig. 4. Shortly after completing the original hand-drawn version of this figure and showing it to Professor Dick Collins, Chair of the technical advisory committee of the National Energy Research, Development and Demonstration Council at that time, Dick commented that I had used all the tricks of the trade for the graph, suppressing the zero on the voltage axis to magnify the advantage. In defence, I

pointed out that I had suppressed the zero on the other axis as well.

Actually, since Voc depends logarithmically on recombination caused by cell deficiencies, each 20mV gain represents a doubling of cell quality, so that graph does suggest, at least in this respect, the magnitude of the advantage UNSW was establishing. Having gained a clear lead in Voc, our focus then became to convert this into an efficiency advantage, by putting into place technology such as double layer antireflection (AR) coatings and fine linewidth, plated metallisation required to capture the full current output capability of the cell.

Figure 5: Team responsible for the world's first 18% efficient cell in 1983 (right to left): Ted Szpitalak (Professional Officer); Andrew Blakers and Stuart Wenham (author's 2nd and 3rd PhD students), Martin Green (author); Jiqun Shi (visiting Chinese scholar, one of the first to benefit from Deng Xiaoping's policy changes); and the late Erik Keller (Professional Officer).

We achieved this in 1983, when we made and had certified the world's first 18% efficiency silicon cell (Blakers et al., 1984; Green et al., 1984a). The elation of the team achieving this result is captured in Fig. 5. Within a few months, with a slight change in cell design, we increased this to an efficiency then certified as above 19% (Green et al., 1984b) (18.4% by present standards).

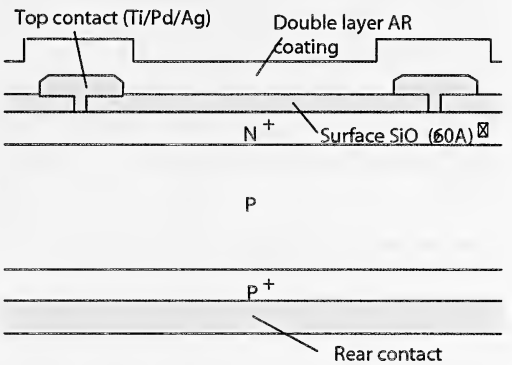

Figure 6: Passivated Emitter Solar Cell (PESC), the first silicon cell with efficiency certified as above 19%.

The new PESC cell structure (Passivated Emitter Solar Cell, with "emitter" referring to the top n^+-doped layer) is shown in Fig. 6. The reason for its high performance was the relatively high cell Voc (651 mV), as might have been expected from our previous work. Two cell features, first identified in our earlier work, were responsible for this high Voc. One was the use of a thin layer of oxide along the top surface of the cell, a vestige of our initial work on tunnelling MOS oxides. This oxide was shown to be crucial for our high voltages by reducing the electronic activity ("passivating") of defects normally associated with the silicon surface. The second was the way contact was made through pre-patterned holes in this oxide, giving small area contacts. This small-area contact was to reduce the impact of the poor electronic properties at the associated metal/silicon interface, an approach the author had earlier proposed in the first paper he wrote after joining UNSW (Green, 1975).

Ideas for future work were outlined in both a grant final report (Green et al., 1984c) and a grant proposal (Green, 1984d) prepared around this time (late 1983). One idea was to

texture the top surface of the cell as in Fig. 3, to reduce reflection and to couple the light obliquely into the cell, allowing it to be absorbed more strongly. The second was to apply similar ideas to those that had proved successful on the top cell surface to the rear of the cell to form the variant of the PERC cell (Passivated Emitter and Rear Cell) shown in Fig. 7.

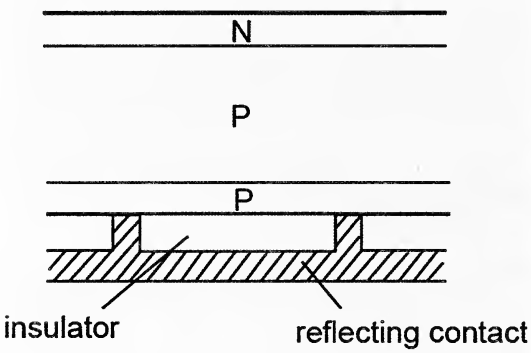

Figure 7: PERC (Passivated Emitter and Rear Cell) as originally proposed in 1983 (Green et al., 1984c; Green, 1984d).

The surface texturing strategy was more quickly implemented, producing the first 20% efficient cell in 1985 (Green et al., 1985; Blakers et al., 1986). This efficiency had earlier been proposed as a practical limit on silicon cell efficiency, becoming the "four-minute mile" of the photovoltaic field. The earlier successes leading to this landmark result had allowed the team to grow by this stage, as apparent from the photograph of the successful 20% team (Fig. 8).

The work then headed off in two different directions. In one strand, we pushed on towards higher efficiency, based on the author's earlier prediction that 25% efficiency was a realistic practical target (Green, 1984e). In the other strand, we explored ways that our 20% PESC cell could be fabricated at low cost. The challenge was to find a way of implementing the cell's fine features, specifically the small area contacts and narrow metal lines, without calling upon expensive photolithographic techniques developed for microelectronics (but used in silicon space cells by this time and in our 20% device).

Figure 8: Photograph of the team achieving the photovoltaic "four-minute mile", the first 20% efficient silicon cell, in 1985. From right to left: Stuart Wenham (author's 3rd PhD student); Jianhua Zhao (5th PhD student); Mike Willison (Professional Officer); Chee Mun Chong at rear (7th PhD student); Martin Green at front (Team Leader); Andrew Blakers (2nd PhD student); Mohan Narayanan at rear (6th PhD student); Ted Szpitalak and Michael Taouk (Professional Officers).

The buried contact solar cell of Fig. 9 was the end result of this work. With funds from NASA-Lewis, we had purchased a second-hand laser scribing system that came with a logbook showing it had already lived a very full life dicing up microelectronic chips in the

USA throughout the 1970s. Our initial aim was to use it to cut our finished cells from the silicon wafers in which they were fabricated. Stuart Wenham converted the system to computer control, giving it the flexibility to scribe previously inaccessible patterns.

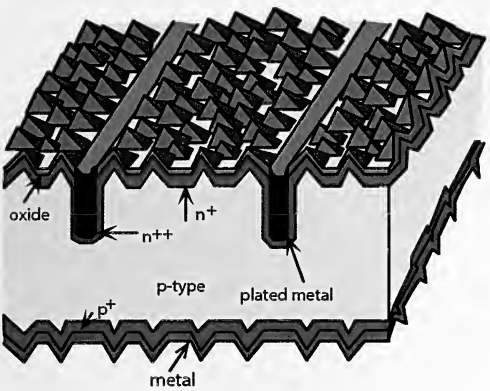

Figure 9: The buried contact solar cell, invented in 1984 (Wenham and Green, 1984).

Initially, we explored use of the laser to nucleate texturing (Wenham and Green, 1983). This stimulated the conception of the buried contact solar cell, a technology subsequently licensed to several companies. The first commercial sales under licence were by AEG Telefunken, with this company supplying buried contact cells to the "Spirit of Biel", a solar car that blitzed the field in the 1990 World Solar Challenge, the solar car race from Darwin to Adelaide. This race had been conceived by Hans Tholstrup as a way of promoting electric vehicle development.

The most successful licensee however was BP Solar, with the company marketing cells under the Saturn product name from 1991-2006 (Mason et al., 2004). Shortly after ceasing this production, BP withdrew from photovoltaic manufacturing entirely. Product valued at over $1 billion was sold under licence to UNSW over this period.

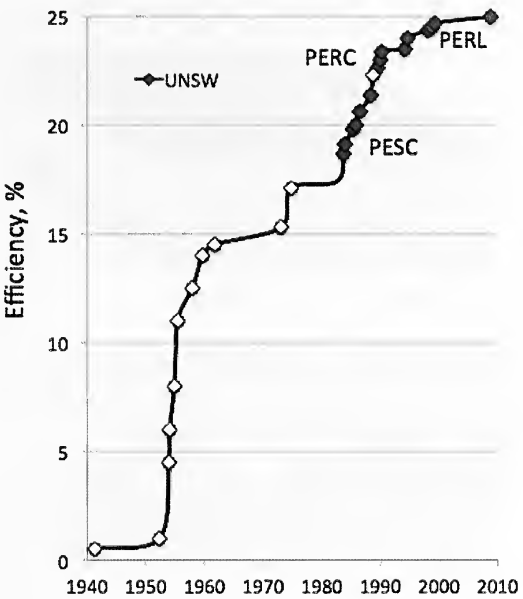

Figure 10: Evolution of silicon laboratory cell efficiency.

In parallel, development of the PERC cell continued, with 21.8% efficiency certified by Sandia National Laboratories in October 1988. The first journal publication appeared the following year (Blakers et al., 1989). Progressive refinement of the PERC structure gave incremental improvements in efficiency (Fig. 10), leading eventually to 24.7% efficiency in 1999 (Zhao et al., 1999), increasing to 25.0% efficiency after a recalibration in 2008 (Green, 2009a), using the PERC/PERL cell structure of Fig. 11.

Although the PERC nomenclature was used originally at UNSW to denote devices with no heavily doped region under the rear contact, the designation is now being used to denote a broader range of devices, including devices that would be classified as PERL devices under the original UNSW nomenclature.

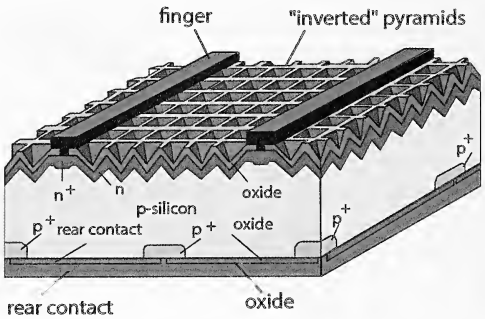

Figure 11: PERC/PERL (Passivated Emitter, Rear Locally-Diffused) solar cell, the first silicon cell to exceed 25% efficiency.

Since then, two other cell structures have shown themselves capable of comparable efficiency. One is the rear junction cell (Fig. 12), originally developed by Stanford University in the mid- to late-1980s and subsequently commercialised by SunPower. This is an unusual cell structure in that both polarity contacts are located on the cell rear. For successful operation, the approach requires high quality wafers and excellent passivation of both front and rear surfaces. Stanford's success in achieving this was instrumental in the subsequent development of PERC cells.

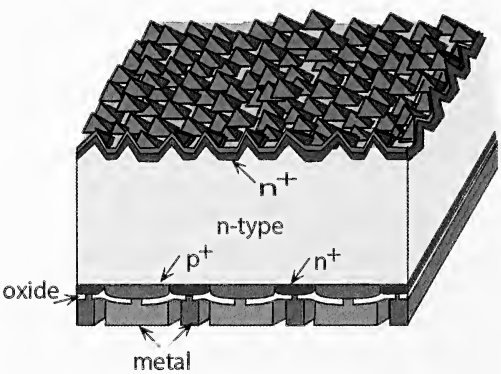

Figure 12: Rear junction solar cell first commercialised by SunPower circa 2004.

The second cell structure (Fig. 13) is the HIT cell (Heterojunction with Intrinsic Thin-layer) developed by Panasonic. The cell takes advantage of the higher energy bandgap in hydrogenated amorphous silicon (a-Si), with the heterojunction formed with crystalline silicon (c-Si) allowing even better surface passivation than the oxides developed at UNSW. However, these a-Si layers are optically more absorbing, blocking some of the incident photons from reaching the c-Si regions of the cell.

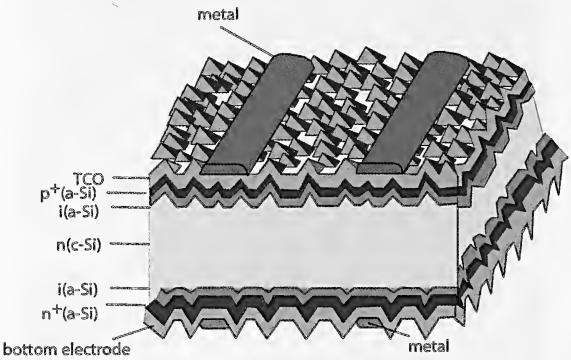

Figure 13: HIT cell commercialised by Sanyo (now Panasonic) circa 1996.

Unlike the PERC cell, both these rear junction and HIT cell structures require high quality wafers to work effectively (carriers have to diffuse from the front to the rear junctions, in one case, while good quality wafers are needed to generate the high voltage required in the HIT cell to offset the reduced coupling of photons into the c-Si substrate).

In 2014, by combining the HIT and rear junction structures, Panasonic was able to inch past the UNSW 25.0% result with 25.6% achieved for silicon cell efficiency (Masuko et al., 2014), with this regarded as a significant achievement for Japanese science. At the triennial World Conference of Photovoltaics held in Kyoto in November 2014, there were at least four invited presentations on the new result solicited by the largely Japanese program committee.

Nonetheless, the commercial potential of the rear junction, HIT and the HIT rear junction approach is limited both by the high costs of the associated processing and the need for specialised wafers (ITRPV, 2015). Products based on the first two of these approaches are presently sold at a premium for the ultra-high efficiency niche market for solar modules.

Crystalline silicon on glass (CSG) cells

Throughout the 1980s and 1990s, the prevailing view within the photovoltaic industry was that silicon wafer-based technology was too material-intensive to have the same low cost potential as a thin-film technology, where a thin active semiconductor layer is deposited on a supporting layer, usually glass.

The author's view, then as now, was that the key thin-film contenders, a-Si, CdTe and CIGS (copper-indium-gallium-selenide), had serious limitations in terms of attainable efficiency for a-Si and the use of toxic and/or scarce elements for CdTe and CIGS. Our work on trapping light into our high efficiency c-Si cells, combined with our ability to effectively passivate surfaces, convinced us that cells based on thin films of polycrystalline silicon were capable of high efficiency. This work was given a further boost when we conceived a parallel multi-junction approach (Green and Wenham, 1994) that would significantly reduce requirements on the material quality required for efficient thin-film silicon cells.

This stimulated a five-year program starting in 1995 supported by Pacific Power, then Australia' s largest power utility company, to bring this technology to commercial readiness. A spin-off company, Pacific Solar, was formed and established a pilot-line for the new technology. By the end of the five-year program, Pacific Power, originally scheduled to finance the following commercialisation phase, had been disbanded and new partners were required. This process resulted in a successor company, CSG Solar, being formed supported largely by Q-Cells, a German company which subsequently became the world's largest photovoltaic manufacturer.

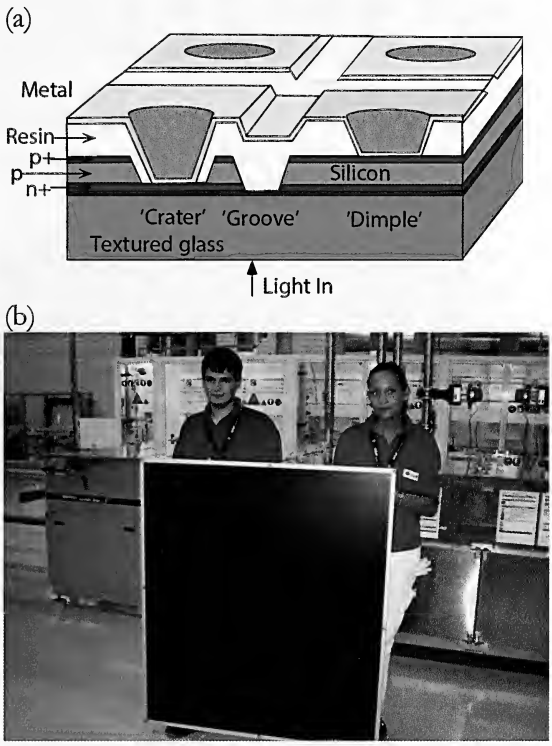

Figure 14: (a) Crystalline silicon on glass (CSG) solar cell with a thin polycrystalline silicon layer 1-2 microns thick deposited onto glass;
(b) 1.4-m² CSG module of 75-105W output manufactured in Germany from 2006.

CSG thin-film polysilicon modules (Green, et al., 2004; Green, 2009b) were introduced onto the market in June 2006 with an annual production capacity of circa 10 MW/year. This was a period where rapid expansion of the PV industry had increased its demand for

high purity polysilicon source material beyond the ability of the microelectronics industry to supply. The subsequent shortage caused a massive escalation in prices that both provided a window of opportunity for thin-film technologies, as well as encouraging a huge number of new entrants into the polysilicon refinement industry. The long lead-time for building these polysilicon plants, however, kept the thin-film window of opportunity open for several years. During this period, about 10 MW of CSG panels were installed around Germany, often in large fractional- or multi-MW fields, such as Eurishofen and Thalheim.

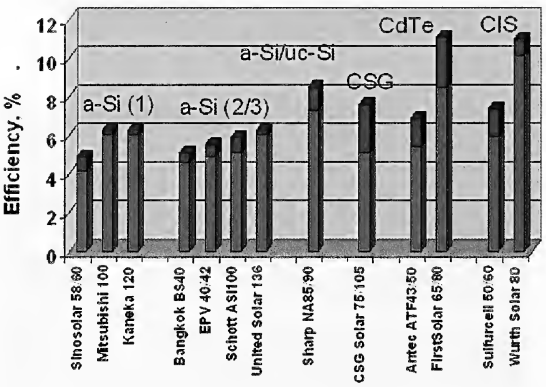

Figure 15: Aspiring thin-film companies listed by Photon International as having product on the market in February 2008. Bars show nominal module efficiency, including range meeting specifications.

However, as new polysilicon refining capacity came on line, the polysilicon shortage quickly turned into a glut and the price of silicon cells dropped precipitously. Of the dozen or so aspiring thin-film companies marketing product in 2008 (Fig. 15), only one (First Solar) was sufficiently well established to survive the onslaught. All others, including CSG Solar, eventually disappeared, although Solar Frontier, with its Cd-free CIGS technology and parents with deep pockets (Shell and Aramco) has since established a viable market position (helped somewhat by the fortuitous timing of the rapid recent growth of a somewhat parochial Japanese photovoltaic market).

The subsequent ongoing reduction in silicon wafer-based manufacturing costs has increased the difficulty of market entry for aspiring thin-film market entrants. The author's present perspective on this is that it is now difficult for any thin-film technology to match silicon's costs, unless the thin-film technology can produce comparable or better energy conversion efficiency. This would be possible with tandem cell stacks, although implementation with CdTe or CIGS technology does not seem imminent.

Market Transition to PERC Cells

As previously mentioned, the cell structure of Fig. 3, first fabricated in 1974 prior to the impact of UNSW research, has been the prototype for most past solar cell production (referred to as the "Al-BSF" approach). The UNSW "buried contact" cell of Fig. 9, the Panasonic HIT cell of Fig. 13 and the Stanford/SunPower Rear Junction cell of Fig. 12 have been higher efficiency variants that have gained restricted market share, championed by a single manufacturer in each case. Similarly, thin-film technologies have also been restricted to a small and diminishing market share, with only First Solar (CdTe) and Solar Frontier (CIGS) manufacturing in any volume in 2015.

Due largely to improvements in the pastes used to form the contacts and the ability to print increasingly narrow contact fingers, combined with the use of hydrogenated silicon nitride AR layers on top of thin interfacial oxides for top surface passivation, the performance of the standard Al-BSF cells have steadily improved to the stage where 19% efficiency on monocrystalline wafers was

quite common in manufacturing in 2014. To break through the 20% barrier in production, more innovative technology was required.

This has focused mainstream interest on bringing the UNSW PERC cell into production. PERC production capacity has increased rapidly over the past three years as indicated by Fig. 16, accounting for the largest share of new production capacity added over this period (Fig. 17).

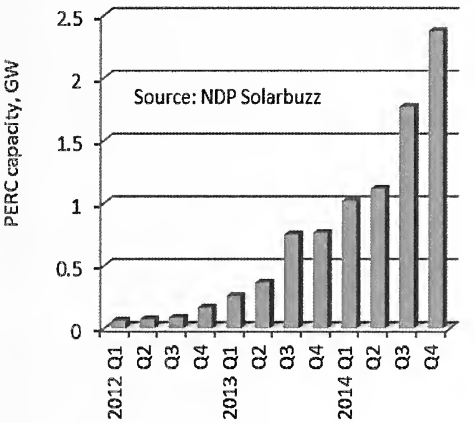

Figure 16: New PERC cell production capability added over recent years (total worldwide capacity for all cell types estimated as 50 GW at end 2014; 1 GW is the output of a large nuclear or coal fired power station) (Data Source: NDP Solarbuzz, Oct. 2014).

Although the PERC cell involves more processing steps than the Al-BSF approach and it is therefore more expensive to make each cell, this extra cost is largely negated by the time the cells are encapsulated into modules, since each module gives extra power output. When installed into the system, the same leveraging results in lower costs than with Al-BSF product, since fewer modules need to be installed for the same rating. Moreover, as the technology is scaled and streamlined, the difference in processing costs between PERC and the former standard Al-BSF approach will decrease.

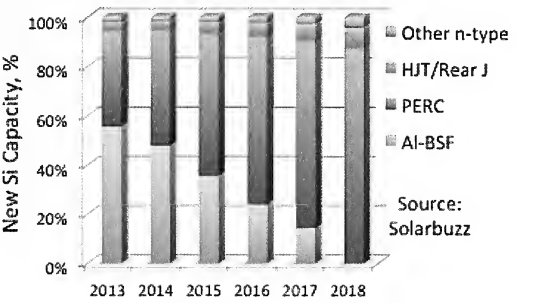

Figure 17: Share of new silicon-based production capacity for different cell approaches (Data Source: NDP Solarbuzz, October 2014).

Consequently, the industry expects that by 2019, UNSW PERC technology will have the largest share of the photovoltaic market and hence leverage the largest share of clean energy investments in solar, estimated as totalling US$149 billion in 2014 (Liebreich, 2015). Figure 18 shows the International Technology Roadmap for Photovoltaics (ITRPV, 2015) estimates for market share of different cell technologies over the coming decade.

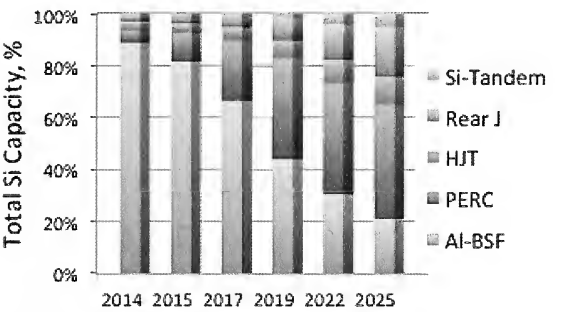

Figure 18: Industry consensus on expected market share of different silicon cell technologies (ITRPV, 2015).

Landmark Achievements

As well as producing the first 20% silicon cell in 1985, several other landmark results have been achieved at UNSW over its 40-year history. In 1989, the group supplied cells to Sandia Laboratories that were used to construct the first photovoltaic system to convert more than 20% of the incident sunlight into electricity, in this case, by focusing the sunlight onto the UNSW cells. In 1993, UNSW repeated this feat for the more challenging case of unconcentrated sunlight. The group also produced the first silicon cell to convert over 20% of space sunlight into electricity, as confirmed by NASA in high-altitude aircraft testing (resulting in a Space Aviation Award to the group). Another 20% first was the first 20% cell on the lower quality multicrystalline silicon that forms the bulk of present commercial production, achieved in 1998 (Green, 2009).

More recent results include the first 25% efficient silicon cell in 2008 (Green, 2009) and, in November 2014, the first photovoltaic system to convert over 40% of incident sunlight into electricity, again by focusing the sunlight (Green et al., 2015). We again hope to duplicate this result in the not too distant future for the more challenging case of unconcentrated sunlight.

Conclusions

The first 40 years of UNSW photovoltaic research have produced some notable research achievements and greatly increased expectations of the efficiency levels that can be attained in commercial production.

Another notable output from the laboratory, only briefly alluded to (Figs. 5 and 8) in the above condensed history, has been the highly trained researchers produced. Many of these have provided the expertise for the transition of the manufacturing industry from high cost regions of the world to the Asia-Pacific region. This, in turn, has being responsible for the dramatic reduction in manufacturing costs over recent years that has positioned photovoltaics as one of the lowest cost options for future electricity production.

To mention only a few of these researchers and their achievements, Stuart Wenham (Figs. 5 and 8), as part-time Chief Technical Officer (CTO) supporting another of my students, Zhengrong Shi (12th PhD student), established the first successful photovoltaic manufacturing venture in China. This was in the form of Suntech Power, which became the world's largest manufacturer, after being the subject of the largest technology float worldwide when the company listed on the New York Stock Exchange in 2005.

Ted Szpitalak (Figs. 5 and 8) headed teams that established the production lines not only at Suntech, but also at JA Solar, China Sunergy and Global Sunrise (Taiwan). Jianhua Zhao (Fig. 8) has been CTO of China Sunergy since 2004, including when the company listed on NASDAQ. Mohan Narayanan (Fig. 5) has had a diverse career in the industry, which included being CTO of Trina Solar, the world's largest manufacturer in 2014, at the time of its initial public offering (IPO). In fact, of the top-5 photovoltaic manufacturers in 2014, all either have former UNSW researchers at CTO or had them at critical stages of the company's development, such as at IPO.

With photovoltaics poised to become one of the largest energy industries of the future, it is hoped that, over the next 40 years, the UNSW laboratory will continue its combined role of blazing the path to future generations of technology while producing researchers

able to spearhead the ongoing growth of the industry.

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References

Adams, W. G. and Day, R. E. (1877) The action of light on selenium, *Proc. Royal Soc.* (London), A25, 113.

Becquerel, A. E. (1839) Recherches sur les effets de la radiation chimique de la lumiere solaire au moyen des courants electriques, *Comptes Rendus de L'Academie des Sciences*, 9, 145-149.

Blakers, A. W., Green, M. A., Shi, J. Q., Keller, E. M., Wenham, S. W., Godfrey, R. B., Szpitalak, T. and Willison, M. R. (1984) 18% efficient terrestrial silicon solar cell, *Electron Device Letters*, 5, 12-13.

Blakers, A. W. and Green, M. A. (1986) 20% efficient silicon solar cell, *Appl. Phys. Lett.*, 48, 215-217.

Blakers, A. W. *et al.* (1989) 22.8% efficient silicon solar cell", *Appl. Phys. Lett.*, 55, 1363-1365.

Brandhorst H. W. (1976) Status of silicon solar cell technology, *NASA Technical Memorandum*, NASA TM X-73531.

Chapin, D. M., Fuller, C. S. and Pearson, G. L. (1954) A new silicon p-n junction photocell for converting solar radiation into electrical power, J. *Appl. Phys.*, 25, 676-677.

Einstein, A. (1905) Über einen die Erzeugung und Verwandlung des Lichtes betreffenden heuristischen Gesichtspunkt, *Annalen der Physik*, 17, 132-148.

Fritts, C. E. (1883) On a new form of selenium photocell, *Proc. American Assoc. for the Advancement of Science*, 33, 97; also, *American J. of Science*, 26, 465.

Green, M. A. (1975) Enhancement of Schottky solar cell efficiency above its semiempirical limit, *Appl. Phys. Lett.*, 27, 287-288.

Green, M. A. and Godfrey, R. B. (1976) MIS solar cell - general theory and new experimental results for silicon, *Appl. Phys. Lett.*, 9, 610-612.

Green, M. A., Blakers, A. W., Shi J. Q., Keller E. M. and Wenham S. R. (1984a) High efficiency silicon solar cells, *IEEE Trans. Electron Devices*, 31, 679-683.

Green, M. A., Blakers, A. W., Shi, J. Q., Keller E. M. and Wenham S. W. (1984b) 19.1% efficient silicon solar cell, *Appl. Phys. Lett.*, 44, 1163-1165.

Green, M. A., Blakers, A. W., Kurianski, J., Narayanan, S., Taouk, M., Szpitalak, T., Wenham, S. R. and Willison, M. R. (1984c) Ultimate performance silicon solar cells, *Final Report, NERDDP Project 81/1264*, Jan. 82 - Dec. 83 (dated Feb. 1984).

Green, M. A. (1984d) High efficiency silicon solar cells, *Proposal in response to RFP RB-4-04033*, SERI (now NREL) (dated March, 1984).

Green, M. A. (1984e) Limits on the open circuit voltage and efficiency of silicon solar cells imposed by intrinsic auger processes, *IEEE Trans. Electron Devices*, ED-31, 671-678.

Green, M. A., Blakers, A. W., Wenham, S. R., Narayanan, S., Willison, M. R., Taouk, M. and Szpitalak, T. (1985) Improvements in silicon solar cell efficiency, *Conf. Record, 18th Photovoltaic Specialists Conference*, Las Vegas, October 1985, (IEEE Publ. No. 85CH2208-7), 39-42.

Green, M. A. and Wenham, S. R. (1994) Novel parallel multijunction solar cell, *Applied Physics Letters*, 65, 2907-2909.

Green, M. A., Basore, P. A., Chang, N., Clugston, D., Egan, R., Evans, R., Ho, J.. Hogg, D., Jarnason, S., Keevers, M., Lasswell, P., O'Sullivan, J., Schubert, U., Turner, A., Wenham S. R. and Young, T. (2004) Crystalline silicon on glass (CSG) thin-film solar cell modules, *Solar Energy*, 77, 857-863.

Green, M. A. (2009a) The path to 25% silicon solar cell efficiency: History of silicon cell evolution, *Prog. in Photovoltaics*, 17, 183-189.

Green, M. A. (2009b) Polycrystalline silicon on glass for thin-film solar cells, *Appl Phys A*, 96, 153-159.

Green, M. A., Keevers, M., Thomas, I., Lasich J. B., Emery K., and King R. R (2015) 40% efficient sunlight to electricity conversion, *Progress in Photovoltaics*, 23, 685-691.

Grondahl, L. O. (1933) The copper-cuprous-oxide rectifier and photoelectric cell, *Rev. Modern Phys.*, 5, 141.

Haynos, J., Allison, J., Arndt, R. and Meulenberg, A. (1974) Photovoltaic Power Generation, *Proc, International Conf.* , Hamburg, ed. H. R. Losch (Deutsche Gesellschaft fair Raumfahrt, West Germany, 1974) p. 487.

ITRPV (2015) *International Technology Roadmap for Photovoltaics*, 6th ed., April 2015.

Liebreich, M. (2015), *Bloomberg New Energy Finance Summit 2015*, New York, 14 April 2015.

Lindmayer, J. and Allison, J. F (1973), *Comsat Technical Review*, 1.

Masuko, K., Shigematsu, M., Hashiguchi, T., Fujishima, D., Kai, M., Yoshimura, N., Yamaguchi, T., Ichihashi, Y., Mishima, T., Matsubara, N., Yamanishi, T., Takahama, T., Taguchi, M., Maruyama, E. and Okamoto, S. (2014) Achievement of more than 25% conversion efficiency with crystalline silicon heterojunction solar cell, *IEEE Journal of Photovoltaics*, 4, 1433-1435.

Mason, N., Artigao, A., Banda, P., Bueno, R. Fernandez, J.M. Morilla, C. and Russell R. (2004) The technology and performance of the latest generation buried contact solar cell manufactured in BP Solar's Tres Cantos facility, *Proc. 19th European PVSEC*, Paris 2004, 2653-2655.

Ohl, R. S. (1941) Light-sensitive electric device", *U.S. Patent No. 2, 402, 622*; Light-sensitive device including silicon, *U.S. Patent No. 2, 443,542*; both filed 27 May, 1941.

Perlin, J. (2013) Let it shine, *New World Library*, California.

Ralph, E. (1975) recent advances in low cost solar cell processing, 11th IEEE Photovoltaic Specialists Conf., 315-316.

Schottky, W. (1938) Halbleitertheorie der Sperrschicht, Naturwissenschaften, 26, 843.

Shockley, W. (1949) The theory of p-n junctions in semiconductors and p-n junction transistors, *Bell Syst. Tech. J.*, 28, 435-489.

Wenham, S. R. and Green, M. A. (1983) Laser grooved solar cell, *Australian Patent 565,214; U.S. Patent 4,626,613, Japanese Patent No. 69933/95.*

Wenham, S. R. and Green, M. A. (1984) Buried contact solar cell, *Australian Patent 570,309; U.S. Patents 4,726,850 and 4,748,130; Taiwanese Patent N1024881; Indian Patent 164492, German Patent P3585697.1-08, South Korean Patent No. 70109.*

Wilson, A. H. (1931) The theory of electronic semi-conductors, *Proc. Royal Soc. A*, 133, 458-491.

Zhao, J., Wang, A. and Green, M. A. (1999) 24.5% efficiency silicon PERT Cells on MCZ substrates and 24.7% efficiency PERL cells on FZ substrates, *Progress in Photovoltaics*, 7, 471-474.

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Long Term Surveys of Pathogen Populations Underpin Sustained Control of the Rust Diseases of Wheat in Australia

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Abstract

The wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*) and wheat leaf rust pathogen *P. triticina* have been present in Australia at least since European colonisation. The stripe rust pathogen of wheat, *P. striiformis* f. sp. *tritici* (*Pst*), was first detected in 1979. Surveys of pathogenic variability in *Pgt* and P. *triticina* began in 1921/22, and of *Pst* in 1979, and have continued annually, uninterrupted ever since. These surveys involve the identification of races (pathotypes) in greenhouse assays, using wheat genotypes ("differentials") carrying different resistance genes. Virulence determinations have targeted principally all stage ("seedling") resistance genes, and only rarely adult plant resistance because of the technical difficulties of working with adult plants under controlled conditions. Data compiled since surveys for each pathogen began strongly to implicate periodic introduction of exotic isolates, single-step mutation, and more rarely somatic hybridisation, as the major processes generating genetic diversity. The surveys have also provided clear evidence of migration of rust isolates throughout Australian cereal growing regions, with many examples of inoculum exchange between the eastern and western cereal belts, principally in a west to east direction. The long term surveys of wheat rust pathogens in Australia have provided both information and pathogen isolates that have underpinned rust control efforts, from gene discovery to post-release management of resistance resources. Increasingly, information on the pathogenicity of rust isolates is being complemented by estimates of genetic diversity, using selectively neutral markers to gain refined insight into the evolution and maintenance of virulence, migration pathways, and periodic long-distance migration events.

Keywords

Biosecurity; genetics; *Puccinia*; resistance; surveillance; *Triticum*

Introduction

The demonstration of pathogenic variation in the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*; *Pgt*) almost 100 years ago by Stakman and Piemeisel (1917), and later in the wheat leaf rust pathogen (*Puccinia triticina*; Mains and Jackson, 1921) and the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*, *Pst*; Gassner and Straib, 1932), were crucial steps in the effort to develop wheat varieties with durable genetic resistance to these important plant pathogens. These early studies identified wheat genotypes ("differentials") that varied in response to rust isolates, and the pathogen variants identified on these standard differentials were referred to as races. Later studies showed that some races could be further subdivided based on pathogenicity on additional differential genotypes, and these sub-types within races

were referred to as strains or pathotypes. Race (pathotype) surveys of the three wheat rust pathogens, which involve greenhouse testing of rust isolates on seedlings of differential genotypes, have since been undertaken in many regions of the world.

In Australia, surveys of the wheat rust pathogens *Pgt* and *P. triticina* were initiated by Professor W. L. Waterhouse at the University of Sydney in 1921/22, and have continued uninterrupted since. Surveys of *Pst* began in 1979 following its first detection in Australia (Wellings, 2007). Professor Waterhouse was President of the Royal Society of New South Wales in 1937, and his Presidential Address published in the Society journal in 1938 was titled "Some aspects of problems in breeding for rust resistance in cereals" (Waterhouse, 1938). In drawing attention to the importance of rust diseases in wheat, it was stated that "the ravages of rust in New South Wales have caused losses amounting to an average amount of £250,000 per annum during the past twenty years". Noting the discovery by Biffen of Mendelian inheritance of resistance to rust in wheat, Waterhouse (1938) further discussed the nature of resistance to rust in wheat, describing three main forms of resistance shown by plants to rust. A summary of the results of pathotype surveys for *Pgt*, *P. triticina*, and several other rust pathogens of oat and barley from 1922 to the end of 1937 were then provided.

Annual surveys of pathogenic variability in the three wheat rust pathogens have continued uninterrupted since 1921/22 (*Pgt* and *P. triticina*) and 1979 (*Pst*), at the University of Sydney. The information generated from this work has been crucial to rust control efforts based on genetics, which have delivered substantial benefits to the Australian economy.

Wheat Rust Pathotype Surveys in Australia

A fascinating feature of some rust fungi is heteroecism; a requirement by some species for two unrelated hosts for completion of the full life cycle. *Pgt* and *Pst* require both wheat (a monocot) and certain species of *Mahonia*, *Berberis*, or *Mahonia* x *Berberis* hybrids (dicots) to complete their full life cycles, while *P. triticina* requires wheat and either *Thalictrum speciosissimum* or *Isopyrum fumaroides* (both dicots). In all three pathogens, sexual recombination occurs on the dicot host, which is often referred to as the alternate host. The alternate hosts of all three rust pathogens are rare or absent in Australia, and it is generally believed that sexual recombination is similarly rare or absent here (Watson and Luig, 1958; Wellings, unpublished). Data compiled since surveys for each pathogen began strongly implicate periodic introduction of exotic isolates, single-step mutation, and more rarely somatic hybridisation, as the major processes generating genetic diversity. The surveys have also provided clear evidence of migration of rust isolates throughout Australian cereal growing regions, with many examples of inoculum exchange between the eastern and western cereal belts, principally in a west to east direction (Park et al., 1995).

Rust resistance gene and pathotype nomenclature

More than 204 resistance genes/alleles have been characterised in wheat to date (Park, 2016). Because of the relatively large chromosome number in hexaploid wheat and the large number of resistance genes already known, new genes are usually mapped to a chromosome arm before being permanently named. Linkage studies and tests of allelism may then be necessary to determine if the locus is distinctive. Once

distinctiveness is proven, the locus is numbered using the designation *Sr* for stem rust, *Lr* for leaf rust, and *Yr* for stripe rust.

Many different systems have been devised to name rust pathotypes. The systems used in Australia were outlined by Park (2008). Pathotypes of *Pgt* are identified following the system described by Watson and Luig (1963) in which six genotypes permit a standard race designation (Stakman *at al.,* 1962), and an additional 11 wheats and two triticales numbered from 1 to 13 (the Australian supplemental differentials) allow further characterisation (Park, 2008). Wheat leaf rust pathotypes are identified using four genotypes that permit a standard race designation (Johnston and Browder 1966), and an additional 11 wheats used as Australian supplementary differentials. Pathotypes of *Pst* are identified using an International differential series and a European series, with a number assigned to each series by the addition of decanery values corresponding to each differential rendered susceptible. The second number is preceded by the letter E to indicate the European series. In Australasia, the pathotype formula is followed by A- or A+ to indicate avirulence or virulence, respectively, for a distinctive resistance present in a selection of the Australian cultivar, Avocet (Wellings *et al.,* 1988) *Puccinia graminis* f. sp. *tritici.*

The population structure of *Pgt* over the past 93 years was strongly influenced by exotic introductions in 1925 (standard race 126), 1954 (standard race 21), and 1969 (standard races 194 and 326) (Table 1), subsequent random mutations to virulence, and selection of genotypes with virulence matching resistance genes in cultivars.

Prior to the detection of race 126 in Western Australia in 1925, Waterhouse (1952) identified six races, differentiated by their virulence/avirulence on 12 differential wheat genotypes used in previous studies in the USA by Stakman and Piemeisel (1917). Studies of the abilities of these six races to infect a wide range of wheat genotypes suggested they belonged to two race groups, one comprising races 43, 44 and 54, and one comprising races 45, 46 and 55 (Waterhouse, 1938). Race 126 spread to eastern Australia and by 1929 had all but superseded the six races detected previously, presumably due to increased aggressiveness (Waterhouse, 1952). The original race 126 along with several derivative pathotypes predominated until 1954, when pathotype 21-0 was first detected, believed to have originated either from *Berberis* in Tasmania (Watson, 1958) or from Africa (Luig, 1977). The frequency of this pathotype increased rapidly in eastern Australia over the next few years, and that of the 126- group declined. Over 50 new pathotypes, all considered to have arisen via step-wise mutations tracing back to pathotype (pt.) 21-0, were detected during the 1950s and 1960s (Luig and Watson, 1970).

Pathotype 34-2,11 was first detected in northern New South Wales in 1957. It combined certain pathogenic (Watson 1981) and isozymic (Burdon et al., 1982) features of both the 126- and the 21- groups, and on this basis was regarded to have arisen via somatic hybridisation between the two groups.

In 1969, two distinct pathotypes (194-1,2,3,5,6 and 326-1,2,3,5,6) were detected for the first time. Both were initially identified from six samples collected during 1969 from New South Wales, Victoria and South Australia, and in 1970, in samples collected from Western Australia (Luig and Watson, 1970). Comparative studies of a range of features suggested that both originated from central Africa, possibly being transported to Australia by high altitude winds across the Indian Ocean (Watson and de Sousa, 1982). The first pathogenic change in these

pathotypes was the development of pt 343-1,2,3,5,6, regarded as a mutant of pt 326-1,2,3,5,6 with added virulence for *Sr5* and detected in 1973 (Watson, 1981). Several other subsequent changes were detected, and members of this group are now well established in Australian wheat growing regions.

Pathotypes of *Pgt* detected in Australia during the past 10 years trace back to pathotypes 21-0, 194-1,2,3,5,6 or 326-1,2,3,5,6 (R.F. Park, unpublished).

Puccinia triticina

The origins and evolutionary relationships between pathotypes of *P. triticina* detected prior to 1980 are not as well understood. Pathogenic variation in *P. triticina* has been monitored since 1926, when Waterhouse (1929) reported two pathotypes that could be differentiated on the Australian wheat cultivar (cv.) Thew (*Lr20*). New virulences were subsequently detected for resistance genes including *Lr3a*, *Lr3ka*, *Lr14a*, *Lr15*, *Lr23* and the complementary genes *Lr27+Lr31*.

Critical surveys of the leaf rust population in Australia between 1980 and 2013 provided strong evidence of five exotic incursions (Table 1), each of which has acted as a founding isolate and given rise to clonal lineages through sequential acquisition of virulence to single resistance genes: pathotype (pt.) 53-1,(6),(7),10,11 (first detected in 1981); pt. 104-2,3,(6),(7),11 (1984); 76-1,3,5,10,12 (1996); 10-1,3,9,10,12 (2006); 76-3,5,9,10 +Lr37 (2006). A sixth lineage, derived from somatic hybridisation between isolates related to pts 53-1,(6),(7),10,11 and 104-2,3,(6),(7),11, was first detected in northern NSW in 1990 (pt. 64-(6),(7),(10),11; Park *et al.*, 1999).

The incursions of five exotic *P. triticina* isolates during this 33 year period are of particular interest given that only one other exotic incursion of a wheat attacking rust was detected during that time (*Pst* in 2002; Table 1). The origin of each, the means by which they were introduced, and just why so many incursions of *P. triticina* have occurred, remain unknown. Also of interest is that while a local mutation to virulence for *Lr13* has not been detected, four of the five incursions carry virulence for this gene (viz. pts 53-1,(6),(7),10,11; 76-1,3,5,10,12; 10-1,3,9,10,12; 76-3,5,9,10 +Lr37). Gene *Lr13* is common in Australian wheat cultivars, and prior to the detection of 53-1,(6),(7),10,11 in 1981, was effective. Following 1981, combinations of *Lr13* with genes such as *Lr1* (e.g. cv. AGT Katana, Arnhem, Diamondbird, Hartog, Kukri), *Lr17b* (e.g. cv. Declic, Lawson, Paterson), *Lr24* (e.g. cv. Naparoo), *Lr26* (e.g. cv. Grebe), and *Lr37* (e.g. cv. Axe, Crusader) remained effective but eventually succumbed either as a result of the subsequent three incursions and/or mutational derivatives of the four *Lr13*-virulent pathotypes.

Puccinia striiformis f. sp. *tritici*

Stripe rust of wheat was first detected in Australia in 1979 (O'Brien et al., 1980). The disease was first detected in Victoria and spread rapidly throughout most of the eastern Australian wheat belt. The pathotype detected in 1979 was identified as 104 E137 A-, and was considered to have been transported to Australia on contaminated clothing, likely from Europe (Wellings, 2007; Table 1). From 1979 to 2006, at least 20 new *Pst* pathotypes were identified in Australia, each presumed single-step mutant pathotypes derived sequentially from the original pathotype detected in 1979 (Wellings, 2007).

Stripe rust was not recorded in Western Australia until August 2002, when it was detected in the Newdegate Shire (Wellings et al., 2003). Analyses demonstrated the

presence of a single pathotype (pt. 134 E16 A+) that was distinct from eastern Australian *Pst* pathotypes not only in pathogenicity but also in AFLP phenotype, indicating a likely exotic origin (Wellings et al., 2003; Table 1). The new pathotype was subsequently detected in eastern Australia (southern New South Wales and South Australia) in September 2003, and has dominated the *Pst* population in all Australian wheat regions surveyed since then (Wellings, 2007). While estimates of the cost of fungicidal control in 2003 were about AUD $43 million, a more severe epidemic developed in 2004 and an estimated AUD $90 million was spent on chemical control (CR Wellings, unpublished data).

Detailed studies of pathotype 134 E16 A+ demonstrated that its virulence profile on specific all stage resistance genes did not pose any greater threat to Australian wheat cultivars. Despite this, many cultivars were noticeably more susceptible to this pathotype at later adult plant growth stages (Wellings and Bariana, 2005). It is generally accepted that pt. 134 E16 A+ is more aggressive than pathotypes related to the 1979 incursion. While studies overseas showed that increased aggressiveness in *Pst* was at least in part attributed to adaptation to higher temperatures (Milus et al., 2006), Australian studies of pt. 134 E16 A+ under controlled conditions failed to show this and suggested that its increased aggressiveness is due to other factors (Loladze et al., 2014). Circumstantial evidence has suggested that this pathotype carries virulence for an uncharacterized APR gene common in current Australian wheat cultivars.

Pathotype Surveys and Rust Control

To have maximum impact in disease control, surveys of pathogenic variability in rust pathogens must be closely integrated with the development and management of new wheat cultivars. Where this has been practised, surveys have provided both information and pathogen isolates that have underpinned rust control efforts, from gene discovery to post-release management of resistance resources. Information generated by pathotype surveys has been used to devise breeding strategies, inform selection of the most relevant isolates for use in screening and breeding, define the distribution of virulence and virulence combinations, allow predictions of the effectiveness/ineffectiveness of resistance genes, and issue advance warning to growers by identifying new pathotypes that overcome the resistance of cultivars before they reach levels likely to cause significant economic damage.

Rust resistance genes in wheat

In 1938, the year Waterhouse's Presidential address to the Royal Society was published, the first stem rust resistant wheat was released in Australia - cv. Eureka. It is now known that Eureka is protected from *Pgt* by the single resistance gene *Sr6*, located on wheat chromosome 2DS. Since then, many loci conferring resistance to the three rust pathogens of wheat have been catalogued: 62 genes/alleles at 55 loci conferring resistance to *Pgt* (*Sr2* – *Sr58*, the symbols *Sr1*, *Sr3*, *Sr4* and *Sr9c* were abandoned due to a lack of reference stocks or duplication with previously described loci); 73 resistance genes/alleles conferring resistance to *P. triticina* (*Lr1* to *Lr73*; the symbols *Lr4*, *Lr5*, *Lr6*, *Lr7*, *Lr8*, *Lr40*, *Lr41* and *Lr43* were abandoned due to a lack of reference stocks or duplication with previously described loci); and 69 genes/alleles at 65 loci (*Yr1* – *Yr67*; *Yr26* is considered to be synonymous with *Yr24*) conferring resistance to *Pst* (Park, 2016).

While most rust resistance genes identified in wheat confer resistance at all growth stages (often referred to as seedling or major genes), some confer resistance only at post-seedling growth stages (often referred to as adult plant resistance (APR) genes). Genes conferring all stage resistance have a major effect on the resistance phenotype when challenged with an avirulent isolate, and consequently have also been referred to as "major genes". APR genes on the other hand can have either major, or more frequently minor, effects on the resistance phenotype, and are accordingly often referred to as "minor genes".

Establishing the genetic relationships between loci conferring rust resistance and their effectiveness has been crucial in gene deployment, ensuring optimal protection of crops from rust infection. This research has relied on the use of rust isolates with defined virulence/avirulence attributes, which have come from pathotype surveys. One important outcome from this research has been the demonstration that some loci comprise alleles that cannot be combined in a homozygous state by hybridisation. Three of 55 loci conferring resistance to *Pgt* are known to comprise allelic series, *viz. Sr7* (alleles *Sr7a* and *Sr7b*), *Sr8* (*Sr8a*, *Sr8b*), and *Sr9* (*Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9f*, *Sr9g*, *Sr9h*) (Park, 2016), three loci conferring resistance to *Pst* comprise multiple alleles (*viz.* three alleles at the *Yr3* locus, two at the *Yr4* locus, and recently *Yr5* and *Yr7* were shown to be allelic), and allelic variation has been recorded at four loci conferring resistance to *P. triticina* (*Lr2*, *Lr3*, *Lr17* and *Lr22*).

Long term studies of pathogenic variability in Australia have also identified several resistance genes for which virulence has not been detected, including three pleiotropic loci that confer APR to all three rust pathogens and to the powdery mildew pathogen *Blumeria graminis* f. sp. *tritici*: *Lr34/Yr18/Sr57/Pm38* (Spielmeyer et al., 2005); *Lr46/Yr29/Sr58/Pm39* (Lillemo et al., 2008); *Lr67/Yr46/Sr55/Pm46* (Herrera-Foessel et al., 2014). All three loci are associated with the morphological trait leaf tip necrosis. A fourth slow rusting APR locus, *Sr2/Yr30*, confers resistance to stem rust and stripe rust and is completely associated with the morphological trait pseudo black chaff (Singh et al., 2005). With the exception of the *Lr67/Yr46/Sr55/Pm46* locus, all have been used widely in commercial wheat varieties over many years and to date no cases of virulence have been reported from pathogenicity surveys.

The value of rust resistance breeding as exemplified by stem rust

Resistance genes have been used to great effect in protecting Australian wheat crops from rust. In estimating the current and potential costs of diseases of wheat in Australia, Murray and Brennan (2009) calculated that rust control through resistance breeding, cultural methods and chemical intervention saved growers more than $1.5 billion per year, of which about 67% ($1 billion) was due to resistance breeding. The biggest impact of resistance breeding in control was estimated for stem rust, accounting for about 93% of the overall value of control estimated at $470 million per year.

Much of the success in controlling stem rust in Australia, especially in the rust-prone region of north-eastern Australia, has been based on exploiting knowledge of pathogen variation in the development of new wheat cultivars. From 1919 to 1938, wheat cultivars were susceptible to the six races detected by Waterhouse (1938). Attempts to breed for resistance to these six races involved crosses between two wheats bred by William Farrer, Canberra and Thew, each carrying different

stem rust resistance genes. A line selected from this cross yielded cv. Euston, which combined the resistances of the two parents and was resistant to the six rust races. Euston was however rendered susceptible by the arrival of race 126 in 1925 and was never grown commercially (Watson and Butler, 1984).

The release of Eureka in 1938 heralded a second phase in stem rust resistance breeding (1938 to 1964) during which cultivars with single genes for resistance were released and new pathotypes with corresponding virulence were detected. Following its release in 1938, cv. Eureka increased in popularity and by 1945 occupied about 18% of the wheat area in northern New South Wales and Queensland (Watson and Luig, 1963). Virulence for *Sr6* was first detected in 1942, and its frequency in the *Pgt* population in this region increased as the area sown to Eureka increased (Watson and Luig, 1963). Examples of other resistance genes that succumbed to presumed mutations in *Pgt* over subsequent years include *Sr11* (cv. Gabo, released in 1942, virulence first detected in 1948), *Sr17* (cv. Warigo, released in 1943, virulence first detected in 1959), and *Sr9b* (cv. Dowerin, released in 1948, virulence first detected in 1960) (Park 2007).

The loss of resistance in cultivars with single gene resistance during this time led to the concept of combining resistance genes to bestow greater durability, proposed by researchers more than 50 years ago (e.g. Watson and Singh, 1952). From 1965 onwards, cultivars with multiple resistance genes were deployed in many regions of Australia, significantly reducing yield losses due to stem rust epidemics. During this third phase and until now, cultivars were protected by resistance genes *Sr2*, *Sr9g*, *Sr12*, *Sr13*, *Sr17*, *Sr22*, *Sr24*, *Sr26*, *Sr30*, *Sr36*, and *Sr38*, singly or more commonly in combinations.

Overall inoculum levels and pathotype diversity in *Pgt* have declined in all wheat-growing regions since the mid-1970s, likely as a consequence of the release of cultivars with such resistance gene combinations. For example, while some 40 pathotypes of *Pgt* were identified in 1973, only 14 pathotypes were detected for the 10-year period 2003–2013 (Zwer *et al.*, 1992; R.F. Park, unpublished).

Concluding Comments, Future Challenges and Directions

Exotic rust threats

The frequency of exotic incursions of wheat rust isolates into Australia has increased since the first such incursion was detected in 1925 (Table 1), presumably due to inadvertent human-mediated transport of rust spores associated with increased and more rapid international travel. Efforts to breed for resistance to rust have been successful in pre-empting local mutations to virulence (McIntosh and Brown, 1997), but less successful in dealing with exotic incursions of rust isolates due to the inability to anticipate when these will occur, from where they will come, and consequently the virulence of any isolates that are introduced.

The only wheat-attacking rust pathogen not present in Australia is leaf rust of durum wheat caused by a variant of *P. recondita* that has *Anchusa* as its alternate host (Anikster et al. 1997). While this pathogen is considered a serious exotic threat, new pathotypes of the three rust pathogens already in Australia also pose serious threats, as clearly shown by the impact of the incursions of new pathotypes of *Pgt* in 1925, 1954 and 1969, and of *Pst* in 2002. Of particular significance in this regard is "Ug99", detected in Africa in 1999 following the observation of severe stem rust infection in wheat nurseries in Uganda in

1998. Greenhouse assays of a single sample of stem rust collected from these nurseries (accession "Ug99") in South Africa identified the presence of a *Pgt* pathotype that has since become known widely as "Ug99" and has the North American race designation TTKSK (Pretorius et al., 2000; Jin et al., 2009). This pathotype overcomes many of the resistance genes that protect wheat cultivars from stem rust, including *Sr31*, a gene for which virulence had not been previously detected. Analyses carried out on samples of stem rusted wheat collected from across a wide area have since shown that pathotype TTKSK is a member of a family of closely related pathotypes that is now known as the "Ug99" lineage. In addition to Uganda, one or more of these pathotypes are present in Eritrea, Ethiopia, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Yemen and Zimbabwe (Park et al., 2011; Singh et al., 2011). The "Ug99" lineage comprises at least seven pathotypes that differ for virulence on resistance genes *Sr21*, *Sr24*, *Sr31* and *Sr36* (Jin et al., 2009). Studies using DNA-based microsatellite markers showed that many of these pathotypes have identical fingerprints, consistent with them being recently derived from a common ancestor via single-step mutation (Visser et al., 2009; 2011). Significantly, surveys in Europe, Turkey, Pakistan and India over recent years have failed to detect any of these pathotypes.

The detailed knowledge of variability in *Pgt* in Australia that has come from pathogenicity surveys over the past 93 years has permitted a detailed understanding of *Sr* genes present in Australian wheat cultivars. This has allowed predictions concerning the responses of these cultivars to exotic threats such as "Ug99" that have been refined by field testing germplasm in Kenya with the assistance of the Kenyan Agricultural Research Institute from 2005-07. Because *Sr31* has not been used widely in Australia, the greatest impact of "Ug99" on germplasm to date has been due to virulence for *Sr30*, combined virulence for *Sr38* with other genes, and more recently, virulence for *Sr24* and *Sr36*. While virulences for *Sr30*, *Sr36* and *Sr38* have been detected in Australia, virulence for *Sr24* has not. The genes *Sr2*, *Sr12*, *Sr13*, *Sr22* and *Sr26*, effective against "Ug99" and derivatives, are important contributors to the resistance present in current Australian wheat germplasm.

Maintaining and improving current levels of rust control

It has been estimated that 50% of the cost of plant improvement involves breeding to maintain current yield and quality levels to meet the challenges of degrading growing environments and evolving pathotypes of major pathogens ("maintenance breeding"; McIntosh and Williamson 2004). Despite the low levels of stem rust over the past 35 years in Australia, *Pgt* remains a serious potential threat to wheat production. The development of virulence for *Sr38* in Western Australia in 2001 (Park, 2008) was a timely reminder in this regard. Despite the substantial successes of resistance breeding in controlling rust diseases in Australian wheat crops, the diseases continue to impact on production, with annual losses estimated at $147 million in 2009, most of which ($127 million) was attributed to stripe rust (Murray and Brennan, 2009).

Protecting the *ca.* $1 billion savings to the Australian wheat industry from resistance breeding and reducing the current impact of rust diseases will only be possible if resistance remains a priority in breeding programs, and if the wheat industry as a whole continues to support genetic approaches to rust control.

Achieving durable resistance to rust diseases

The concept of durable resistance was introduced about 40 years ago, following a breakdown in the slow rusting or APR of several English winter wheats to stripe rust, including Joss Cambier, and the continued effectiveness of resistance in several other cultivars including Cappelle Desprez and Hybrid de Bersee. The resistance in the latter was referred to as durable, and durable resistance defined as "resistance that remains effective when a cultivar is grown widely in environments favouring disease development" (Johnson 1978). Durable resistance is a descriptive term; it does not provide any explanation of the causes underlying long-lasting resistance. It does, however, contain two conceptual elements, one being that there may be any of several underlying causes for durable resistance and the other that resistance that has remained effective for a long period of widespread use may not necessarily continue to do so in the future (Johnson, 1984).

Experience to date suggests that combinations of multiple effective resistance genes contribute to durability by lowering the chance of virulence matching gene combinations developing. Furthermore, some genes, notably the pleiotropic APR genes *Lr34/Yr18/Sr57/Pm38*, *Lr46/Yr29/Sr58/Pm39* and *Sr2/Yr30*, appear to be intrinsically durable. These pleiotropic APR genes have in many cases been used to great effect in conjunction with all stage resistance genes, providing backbone resistance that may also have contributed to increased durability of all stage resistance genes.

The advent of molecular genetics has provided new tools to assist in developing wheat cultivars with durable resistance. The number of resistance genes for which tightly linked high throughput DNA markers are available continues to increase, allowing marker assisted selection and greater efficiency in assembling gene combinations (Kuchel et al., 2007). The ability to incorporate multiple resistance genes by transformation will also become possible as more rust resistance genes are cloned. To date, genes/alleles at seven rust resistance loci have been cloned from wheat: three conferring resistance to leaf rust (*Lr1*, Cloutier et al., 2007; *Lr10*, Feuillet et al., 2003; *Lr21*, Huang et al., 2003), one conferring resistance to stripe rust (*Yr36*, Fu et al., 2009), two conferring resistance to stem rust (*Sr33*, Periyannan et al., 2013; *Sr35*, Saintenac et al., 2013), and one conferring resistance to all three rusts and to powdery mildew (*Lr34/Yr18/Sr57*, Krattinger et al., 2009). An important consideration in this approach is, however, possible suppression of resistance genes. For example, recent studies showed that the powdery mildew susceptibility allele in wheat *Pm3C*, and three resistance alleles (*Pm3a*, *Pm3b* and *Pm3f*) all suppressed the resistance gene *Pm8* (Hurni et al., 2014). The mechanism of suppression was related to a post-translational mechanism: direct interaction between the two proteins produced by the resistance genes in tobacco suggested that the formation of a heteromeric protein complex may interfere with signal transmission in the defence reaction. While this example may be an exception, clearly any strategy based on combining cloned resistance genes will need to take this into consideration.

Improving rust diagnostics

In concluding his Presidential address to the Royal Society of NSW, Waterhouse (1938) said: "Looking now to the future, from what has been set out it is apparent that specialisation is to be expected in each of the cereal rusts. And it must not be forgotten that changes in the physiological races present

may be looked for as time goes on. Any breeding programme designed to give control of rust should take fully into account this phenomenon of specialisation." These comments have been soundly verified by the work that has been done since on understanding rust pathogen variation, which remains a crucial part of genetic approaches in controlling these pathogens.

The methods developed to identify rust pathotypes have not changed greatly over the past 90 years or so. Using greenhouse assays, it takes about 2 to 3 weeks to identify a pathotype. In addition to providing new tools to expedite wheat breeding, molecular genetics is beginning to provide insights into rust pathogens at the genomic level (e.g. Duplessis et al., 2011). This has included the development of DNA-based molecular diagnostics. Although it is not yet possible to identify a rust pathotype using molecular diagnostics, whole genome sequencing has allowed the development of diagnostic microsatellite (Simple Sequence Repeat; SSR) markers that have allowed rapid placement of rust isolates into clonal lineage genotypes (e.g. Bailey et al., 2015). A preliminary study in which 70 *Pgt* isolates were sequenced has allowed the development of a *Pgt* SNP Chip (Illumina GoldenGate) containing 1,532 SNP, which can genotype an isolate of *Pgt* based on only a single pustule (Szabo et al., 2014). While such techniques are unlikely to replace greenhouse seedling assays, they will undoubtedly be used more routinely to provide rapid information on the identity of prevailing rust isolates as well as a greater understanding of rust pathogen population genetics and the development of new, sustainable approaches to rust control.

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References

Anikster, Y., Bushnell, W.R., Eilam, T., Manisterski, J., and Roelfs, A.P. (1997) *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats, and rye. *Canadian Journal of Botany* 75, 2082-2096.

Bailey, J., Karaoglu, H., Wellings, C.R., and Park, R.F. (2015) PCR-based simple sequence repeat markers for diagnostic identification of major clonal lineages of *Puccinia striiformis* f. sp. *tritici* and related stripe rust pathogens in Australia. *Australasian Plant Pathology* 44, 97-103.

Burdon, J.J., Marshall, D.R., Luig, N.H., and Gow, D.J.S. (1982) Isozyme studies on the origin and evolution of *Puccinia graminis* f. sp. *tritici* in Australia. *Australian Journal of Biological Science* 35, 231–238.

Cloutier, S., McCallum, B.D., Loutre, C., Banks, T.W., Wicker, T., Feuillet, C., Keller, B., and Jordan, M.C. (2007) Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. *Plant Molecular Biology* 65, 93–106.

Duplessis, S., Cuomo, C.A., Linc, Y.C., Aertsd, A., Tisseranta, E., Veneault-Fourreya, C., Jolye, D.L., Hacquard, S., Amselem, J., Cantarel, B.L., Chiu, R., Coutinho, P.M., Feau, N., Field, M., Frey, P., Gelhaye, E., Goldberg, J., Grabherr M.G., Kodira C.D., Kohler, A., Kües, U., Lindquist, E.A., Lucas, S.M., Mago, R., Mauceli, E., Morin, E., Murat, C., Pangilinan, J.L., Park, R., Pearson, M., Quesneville, H., Rouhier, N., Sakthikumar, S., Salamov, A.A, Schmutz, J., Selles, B., Shapiro, H., Tanguaye, P., Tuskand, G.A., Henrissat, B., Van de Peer, Y., Rouzé, P., Ellis, J.G., Dodds, P.N., Schein, J.E., Zhong, S., Hamelin,

R.C., Grigoriev, I.V., Szabo, L.J. and Martin, F. (2011) Obligate biotrophy features unravelled by the genomic analysis of rust fungi. *Proceedings of the National Academy of the USA* 108, 9166-9171.

Feuillet, C., Travella, S., Stein, N., Albar, L., lie Nublat, A., and Keller, B. (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proceedings of the National Academy of Sciences USA* 100, 15253-15258.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., and Dubcovsky, J. (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science* 323, 1357-1360.

Gassner, G., and Straib, W. (1932) Die bestimmung der biologischen rassen des weizengelbrostes (*Puccinia glumarum* f. sp. *tritici* (Schmidt.) Erikss. und Henn.). *Arbeiten der Biologischen Reichsanstalt für Land und Forstwirtschaft, Berlin* 20, 141-163.

Herrera-Foessel, S.A., Singh R.P., Lilemo M., Huerta-Espino J., Bhavani S., Singh, S., CaiXia, L., Clavo-Salazar, V., and Lagudah, E.S. (2014) *Lr67/ Yr64* confers adult plant resistance to stem rust and powdery mildew in wheat. *Theoretical and Applied Genetics* 127, 781-789.

Huang, L., Brooks, S.A., Li, W., Fellers, J.P., Trick, H.N., Gill, B.S. (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics* 164, 655–664.

Hurni, S., Brunner, S., Stirnweis, D., Herren, G., Peditto, D., McIntosh, R.A., and Keller, B (2014) The powdery mildew resistance gene *Pm8* derived from rye is suppressed by its wheat ortholog *Pm3*. *The Plant Journal* doi: 10.1111/tpj.12593.

Jin, Y., Szabo, L.J., Rouse, M.N., Fetch, T., Pretorius, Z.A., Wanyera, R., Njau, P. (2009) Detection of Virulence to Resistance Gene Sr36 Within the TTKS Race Lineage of *Puccinia graminis* f. sp. *tritici*. *Plant Disease* 93, 367-70.Johnston, C.O., and Browder, L.E. (1966) Seventh revision of the international register of physiologic races of *Puccinia recondita* f. sp. *tritici*. *Plant Disease Reporter* 50, 756-760.

Johnson, R. (1978) Practical breeding for durable resistance to rust diseases in self-pollinating cereals. *Euphytica* 27, 529–540.

Johnson, R. (1984) A critical analysis of durable resistance. *Annual Review of Phytopathology* 22, 309-330.

Krattinger, S.G., Lagudah, E.S., Spielmeyer, W., Singh, R.P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L.L., and Keller, B. (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 323, 1360-1363.

Kuchel, H., Fox, R., Reinheimer, J. et al. (2007) The successful application of a marker–assisted wheat breeding strategy. *Molecular Breeding* 20, 295–308.

Lillemo, M., Asalf, B., Singh, R.P. Huerta-Espino J., Chen X.M., He, Z.H., and Bjornstad A. (2008) The adult plant resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. *Theoretical and Applied Genetics* 116, 1155-1166.

Loladze, A., Druml, T., and Wellings, C.R. (2014) Temperature adaptation in Australasian populations of *Puccinia striiformis* f. sp. *tritici*. *Plant Pathology* 63, 572-580.

Luig, N.H. (1977) The establishment and success of exotic strains of *Puccinia graminis tritici* in Australia. *Proceedings of the Ecological Society of Australia* 10, 89–96.

Luig, N.H., and Watson, I.A. (1970) The effect of complex genetic resistance in wheat on the variability of *Puccinia graminis* f. sp. *tritici*. *Proceedings of the Linnean Society of New South Wales* 95, 22–45.

Luig, N.H., Burdon, J.J. and Hawthorn, W.M. (1985) An exotic strain of *Puccinia recondita* in New Zealand. *Canadian Journal of Plant Pathology* 7, 173-176.

Mains, E.B., and Jackson, H.S. (1921) Two strains of *Puccinia triticina* on wheat in the United States. *Phytopathology* 11, 40 (abstr.).

McIntosh R.A., and Brown G.N. (1997) Anticipatory breeding for resistance to rust diseases in wheat. *Annual Review of Phytopathology* 35, 311–326.

McIntosh R.A., and Williamson P. (2004) Cereals: Grain Diseases. In Wrigley, C.H., Corke and

Walker, C.H., eds. *Encyclopaedia of Grain Science*, pp. 228-238. Elsevier Academic Press: Oxford.

Milus, E.A., Seyran, E., and McNew, R. (2006) Aggressiveness of *Puccinia striiformis* f. sp. *tritici* isolates in the south-central United States. *Plant Disease* 90, 847–852.

Murray, G.M., and Brennan, J.P. (2009) The current and potential costs of wheat diseases in Australia. Grains Research and Development Corporation: Canberra Australia. 69 pp.

O'Brien, L., Brown, J.S., Young, R.M., and Pascoe, I. (1980) Occurrence and distribution of wheat stripe rust in Victoria and susceptibility of commercial wheat cultivars. *Australasian Plant Pathology* 9, 14.

Park, R.F. (2007) Stem rust of wheat in Australia. *Australian Journal of Agricultural Research* 58, 558-566.

Park, R.F. (2008) Breeding cereal for rust resistance in Australia. *Plant Pathology* 57, 591–602.

Park, R.F. (2016) Wheat: biotrophic pathogen resistance. *Encyclopedia of Food Grains*. CR Wrigley ed. Elsevier.

Park R.F., Burdon J.J., and McIntosh R.A. (1995) Studies on the origin, spread, and evolution of an important group of *Puccinia recondita* f. sp. *tritici* pathotypes in Australasia. *European Journal of Plant Pathology* 101, 613–622.

Park R.F., Burdon J.J., and Jahoor A. (1999) Evidence for somatic hybridisation in the leaf rust pathogen of wheat (*Puccinia recondita* f. sp. *tritici*). *Mycological Research* 103, 715–723.

Park, R.F., Fetch, T., Hodson, D., Jin, Y., Nazari, K., Prashar, M. and Pretorius, Z.A. (2011) International surveillance of wheat rust pathogens - progress and challenges. *Euphytica*. DOI 10.1007/s10681-011-0375-4.

Periyannan, S., Moore, J., Ayliffe, M., Bansal, U., Wang, X., Huang, L., Deal, K., Luo, M., Kong, X., Bariana, H., Mago, R., McIntosh, R., Dodds, P., Dvorak, J., and Lagudah, E. (2013) The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. *Science* 341,786-788.

Pretorius, Z.A., Singh, R.P., Wagoire, W.W., and Payne, T.S. (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Disease 84, 203.

Saintenac, C., Zhang, W., Salcedo, A., Rouse, M.N., Trick, H.N., Akhunov, E., and Dubcovsky, J. (2013) Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. *Science* 341,783-786.

Singh, R.P., Huerta-Espino, J., and William, H.M. (2005) Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turkish Journal of Agriculture and Forestry* 29, 121-127.

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P.K., Singh, S., and Govindan, V. (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology* 49, 465–481.

Spielmeyer, W., McIntosh R.A., Kolmer J.A., and Lagudah E.S. (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. *Theoretical and Applied Genetics* 111, 731-735.

Stakman, E.C., and Piemeisel, F.J. (1917) Biologic forms of *Puccinia graminis* on cereals and grasses. *Journal of Agricultural Research* 10, 429–495.

Stakman, E.C., Stewart, D.M., and Loegering, W.Q. (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. Agricultural Research Service E617. (United States Department of Agriculture: Washington DC.)Szabo, L.J., Cuomo, C., and Park, R.F. (2014) The stem rust pathogen of grasses, *Puccinia graminis*. In: Genomics of Plant-associated Fungi and Oomycetes (ed R. A. Dean) Springer, pp. 177-196.

Visser, B., Herselman, L., and Pretorius, Z.A. (2009) Genetic comparison of Ug99 with selected South African races of *Puccinia graminis* f. sp. *tritici*. *Molecular Plant Pathology* 10, 213–222.

Visser, B., Herselman, L., Park, R.F., Karaoglu, H., Bender, C.M., and Pretorius, Z.A. (2011) Characterization of two new wheat stem rust races within the Ug99 lineage in South Africa. *Euphytica*. DOI 10.1007/s10681-010-0269-x.

Waterhouse, W.L. (1929) Australian rust studies. I. *Proceedings of the Linnean Society of New South Wales* 54, 615–680.

Waterhouse, W.L. (1938) Presidential address Part I. General. Part II. Some aspects of problems in breeding for rust resistance in

cereals. *Journal and Proceedings of the Royal Society of New South Wales* 72, 1– 54.

Waterhouse, W.L. (1952) Australian rust studies. IX. Physiological race determinations and surveys of cereal rusts. *Proceedings of the Linnean Society of New South Wales* 77, 209–258.

Watson, I.A. (1958) The present status of breeding disease resistant wheats in Australia. *Agricultural Gazette of New South Wales* 69, 630–660.

Watson, I.A. (1981) Wheat and its rust parasites in Australia. In *Wheat Science - Today and Tomorrow,* pp. 129–147. Eds L.T. Evans, W.J. Peacock. Cambridge University Press: Cambridge, UK.

Watson, I.A., and Butler, F.C. (1984) *Wheat Rust Control in Australia. National Conferences and Other Initiatives and Developments.* The University of Sydney: Sydney, Australia.

Watson, I.A., and de Sousa, C.N.A. (1982) Long distance transport of spores of *Puccinia graminis tritici* in the southern hemisphere. *Proceedings of the Linnean Society of New South Wales* 106, 311–321.

Watson, I.A., and Luig, N.H. (1958) Widespread natural infection of barberry by *Puccinia graminis* in Tasmania. *Proceedings of the Linnean Society of New South Wales* 83, 181–186.

Watson, I.A., and Luig, N.H. (1963) The classification of *Puccinia graminis* var. *tritici* in relation to breeding resistant varieties. *Proceedings of the Linnean Society of New South Wales* 88, 235–258.

Watson, I.A., and Singh, D. (1952) The future for rust resistant wheat in Australia. *Journal of the Australian Institute of Agricultural Science* 18, 190–197.

Wellings, C.R. (2007) *Puccinia striiformis* in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979–2006. *Australian Journal of Agricultural Research* 58, 567–575.

Wellings, C.R., and Bariana, H.S. (2005) Wheat variety response to stripe rust. *Cereal Rust Report* **3** (http://www.agric.usyd.edu.au:8888/pbi/cereal_rust_reports_crr.htm)Wellings, C.R., McIntosh, R.A., and Hussain, M. (1988) A new source of resistance to *Puccinia striiformis* f. sp. *tritici* in spring wheats (*Triticum aestivum*). *Plant Breeding* **100**, 88-96.

Wellings, C.R., Wright, D.G., Keiper, F. et al. (2003) First detection of wheat stripe rust in Western Australia: evidence for a foreign incursion. *Australasian Plant Pathology* 32, 321–2.

Zwer, P.K., Park, R.F., and McIntosh, R.A. (1992) Wheat stem rust in Australia 1969 - 1985. *Australian Journal of Agricultural Research* 43, 399–431.Ackoff, R. L. (1975) Does quality of life have to be quantified?; *General Systems,* 20, 213-219.

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The Relationship Between Engineers and Society: is it currently fulfilling its potential?

An Invited Discourse

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Abstract

The structure and operation of the complex societies of the developed world are completely dependent on countless applications of technology, as we can observe in our daily lives. But what we may be less conscious of is that the evolution of society – what it will be tomorrow – is also highly dependent on our development and application of technology; to a large extent our society is what it is today as a result of the technology we chose to apply in the past. Engineers play a major role in the development and application of technology, and so have a responsibility for the evolution of society. It is the nature of that responsibility that is the subject of this paper, and it is suggested that it is mainly in providing the information society requires in order to make its decisions.

Introduction

The mission of the Royal Society of NSW is to encourage studies and investigations in science, art, literature, and philosophy, and of these areas of intellectual activity science is currently by far the dominant topic in the Society's discourse. Science is about knowledge about Nature, and simplistically one could therefore expect that there is nothing further to be said about this knowledge as such; it is either there or it is not. But knowledge is a very human product and involves such concepts as truth, verifiability, acceptability, and many more, so that there is a significant branch of philosophy dedicated to the study of the nature of this knowledge under the umbrella of Philosophy of Science. Besides strictly philosophical issues, this umbrella also covers work that is to a large degree sociology, as it is concerned with how scientists work, how they associate, how they form opinions, etc. However, there is one important concept that does not apply to scientific knowledge, and that is *value*; not in terms of money or potential usefulness, but in an ethical sense. Knowledge itself is neither good nor bad. Knowledge has no influence on anything; it is only the application of knowledge that has an influence and can be good or bad.

The application of scientific knowledge is a central part of engineering, but while there are strong links between the three areas of intellectual activity – science, philosophy, and engineering – the philosophical aspects of engineering are on the whole quite different to those treated in the Philosophy of Science. In both, we can talk about the nature of things themselves (ontology) and the nature of our knowledge of them (epistemology). Engineering raises some special issues, arising mainly from the role of heuristics in engineering practice, but it is above all ethical issues in engineering, either explicitly or

implicitly, that have seen considerable activity from both philosophers and engineers, as evidenced by some recent publications (Hector 2012, Christensen 2007, Beder 1995, Unger 1994, Vann 1997). The issues have been mainly concerned with the behaviour of individual engineers towards other individuals as well as their environments, as exemplified by numerous Codes of Ethics. These form a set of *rules* that define engineering as a practice; they form a framework that restricts *how* engineering is to be performed, but say nothing about the *value* of the engineering. The purpose of this paper is to initiate a discussion about an aspect of the responsibility of engineers that has received relatively little attention. It arises from the accepted realisation that technology has a significant and rapidly increasing influence on the evolution of society. Engineers play a major role in the development and application of technology, and with this role comes a certain responsibility for the direction in which society develops. Some of the issues related to recognising and exercising this responsibility have been the subject of recent work (Aslaksen 2014); this paper focuses on the nature of the responsibility itself. How can it be defined in operational terms, how can it be quantified, how is it influenced by other features of society? To approach these and related issues, the paper first gives an overview of previous work relevant to the relationship between technology and society, to the relationship between technology and engineers, and to the relationship between engineers and society; all three of which are crucial to any discussion of the responsibility of engineers for the evolution of society. The core of the paper is then the development of an understanding of what this responsibility consists of and what its limitations are, and as this understanding is necessarily based on a view of the process of evolution, the disclosure of that view is an important component of the paper.

Background: Technology and Society

The meaning of the word "technology" relates to the field of human activity that may be described as the modification of elements of the natural surroundings in order to meet a need; what we shall call a *purposeful* modification (Aslaksen 2012). It started when humans developed the mental ability to recognise the possibility of such a modification and the physical dexterity to realise it, and the purpose included giving visual pleasure or increasing one's self esteem (painting, ornaments, sculptures), worshipping a deity (monuments, temples), providing shelter (dwellings), increasing mobility (roads, bridges, boats), providing food (traps, weapons, agriculture), preparing, serving, and storing food (bowls, pots, plates), and so on. This is roughly what the ancient Greeks identified as *techné* (which in Greek is spelt τεχνη, and would actually be *texnh* with Latin letters). According to the dictionary (LSJ 1940), the word means "art, skill, cunning of hand", and so, in the broadest sense, applied to any creative activity and the products that arose from it. When engineering became a recognised profession and the subject of philosophical enquiries as to its content and purpose, much of the early work was in the German language, and the word *Technik* was adopted to refer to both the activity of and artefacts produced by engineering. As a result, the word *technology* took on this same meaning in much of the work in the English language on philosophical enquiries related to engineering. But within the engineering profession itself, technology means the knowledge and resource base engineers apply to create new works; the activity of creating the works is called engineering.

The identification of the resource and knowledge bases as constituting "technology" is a deviation from the use of "technology" by philosophers and sociologists, where it is used in a much more encompassing manner, such as "the production and use of artefacts". And many publications on the philosophy of technology make no mention of engineering at all. However, while much of what philosophers say about technology can be reflected onto engineering, it is important to keep the distinction in mind. Whereas philosophers see technology as an activity (or at least including activities) and the resulting artefacts, as e.g. in Li (2010), no engineer would speak of "doing technology".

The concept of "technology" is also used extensively in sociology. The tension between the usage of "technology" in engineering and in philosophy and sociology was discussed briefly in Aslaksen (2013a), but a useful perspective on the everyday use of the concept is given by Leo Marx (Marx 1994), where he shows that the character and representation of "technology" changed in the nineteenth century from discrete, easily identifiable artefacts (e.g. a steam engine) to abstract, scientific, and seemingly neutral systems of production and control. As a result, the newly refurbished concept of "technology" became invested with a host of metaphysical properties and potencies that invited a belief in it as an autonomous agent of social change, attributing to it powers that bordered on idolatry.

The point of this is that the meaning of "technology" is unavoidably context-dependent, and that must be taken into consideration throughout this paper.

The relationship between technology and society has been a subject of study and discussion for more than a century. Heidegger (Heidegger 1977) recognised the achievements of engineering and the benefits of technology, but thought that there were already indications that this force was controlling us, that Nature in itself was losing its value and becoming simply something to be exploited, and that a run-away situation could arise. Dessauer (Dessauer 1956) saw technology (and engineering) as an expression of God's plan for mankind, which would lead us to independence from material restrictions and elevate us to a spiritual level, whereas Ellul (Ellul 1980) essentially saw the force as evil and the evolution of technology as the Devil's work. And, of course, we should not forget how we were banished from Paradise by tasting the forbidden fruit of the tree of knowledge; a parable that makes the engineer's role somewhat akin to that of the snake, tempting society to move ever further away from its "natural" state.

Much of the early work on the influence of technology regarded it as taking part between two separate spheres of existence; a genuine (or intrinsically, or unsullied) human sphere and a sphere in which technology is prevalent, see e.g. Mackenzie (1999) and Smith (1994). Technology was seen as developing under its own imperative, and so the interaction was a one-way process, with conflicts arising at the interface between the two, and with humans sometimes seen as the "victims" of technology. More recent work sees the interaction as a process that is both two-way and so dynamic that it is not possible to make a clear-cut distinction between humans and technology. Human behaviour is always a hybrid of supposedly human and technical aspects, and what is of interest are the different kinds of human-technology interactions. This two-way process is treated in an article by Dorrestijn (Dorrestijn 2012) in the context of an analysis of the relevance of Foucault's work to a philosophy of

technology, and is then reflected in the relationship between technology and society, which together form a complex system. An article by Callon (Callon 1987), in which he describes and analyses the electric car project undertaken by Electricité de France in the 1970s, is an excellent example of this. He introduces the notion of an actor network to account for the interactions between the numerous elements making up the system, and emphasizes that these elements include people, organisations, and social movements, but also technological artefacts and assumptions.

Another approach to investigating the relationship between technology and society is "social experimentation", which consists of introducing an application to a segment of society and observing the effects. It was used in the 70s and early 80s, but its utility was controversial, see e.g. Hausman and Wise (1985) and Archibald and Newhouse (1980). More recently the idea of technology introduction as social experimentation has been revived, in particular with regard to ethical concerns and the public's "right to know", by such groups as the 3TU.Centre for Ethics and Technology (http://ethicsandtechnology.eu).

This two-way process, the mutual interaction between technology and society, can be viewed as a form of supply-and-demand relationship. Society makes demands, in the form of needs and desires; technology provides solutions, and society provides feedback in the form of the degree of acceptance of these solutions. The central issue is on what basis society evaluates the solutions; the quality of the information supplied by the technology providers.

Engineers and Technology

Nowhere is the context-dependence of the meaning of "technology" more apparent than in the relationship between engineers and technology. If by "technology" we understand the resource and knowledge bases, then the relationship is very close; engineers are the creators of technology. The direction and pace of development are influenced by the local market and investment conditions, as well as by advances in science, but the new construction elements, tools, and techniques added to the resource base, and the related knowledge added to the knowledge base in the form of articles, textbooks, and standards are all produced by, and the responsibility of, engineers.

But, if we take the meaning of "technology" to be that given to it by society in general, i.e. by non-engineers, then the relationship of engineering to technology becomes much less clearly defined. What society experiences as "technology" is influenced by many other groups of people besides engineers, as has been pointed out by many authors, e.g. Hughes (1987). The reason for this is that engineers are today almost completely embedded in the framework we call *industry*, which encompasses not only private industry, but also government entities involved in applying technology and educational institutions involved in developing and disseminating technology. What society experiences as technology is the product of industry. An engineer on his or her own can accomplish very little, and so what society sees is the work of the engineer through an industrial interface in which numerous people play a part, such as workers, tradespeople, marketing and sales people, business managers, financiers, etc. This has become more pronounced with the outsourcing of the engineering of public works to private industry, but also as a result of the increased

politicisation of the public service. The position of the engineer within industry, and the relationship between engineers as employees and the industrial entities in which they are employed will be a major boundary condition when it comes to considering the responsibility of engineers to society, and it is not a new issue. In his essay *The Captains of Finance and the Engineers*, Thorstein Veblen wrote; "It is perhaps unnecessary to add the axiomatic corollary that the captains have always turned the technologists and their knowledge to account in this manner (for their own gain) only so far as would serve their own commercial profit, not to the extent of their ability; or to the limit set by the material circumstances; or by the needs of the community" (Veblen 1921).

What is presented to society are applications of technology (in the engineering sense); society just call it "technology" as a sort of shorthand, but without much thought as to what this shorthand all encompasses. The process that leads from an idea in the creator's mind to a product in the user's hand is largely hidden from most of society, and, of particular relevance in the present context, the role and responsibility of engineers in this process are hidden. Most people would have a very vague (if any) idea of how the "technology" they see all around them and use every day is related to the work of engineers. It is paradoxical that as technology becomes more and more pervasive, the relationship of engineers to technology, as seen by society, is becoming less and less visible.

The relationship between engineers and industry, or what society sees as technology, is also discussed as part of a recent essay by Newberry (Newberry 2007). In particular, he makes reference to the suggestion by Noble (Noble 1977) that industry has forcefully shaped the mechanisms for engineering education and professional socialisation in order to produce a "domesticated breed of engineers".

Engineers and Society

The main point of the previous section regarding the significance of the relationship of engineering to society is that this is mainly an indirect relationship, with industry, in its various forms, as the intermediary. While society sees various occupations, from dentist to bus driver, at work and understand what they do, engineers are largely invisible. That is, the picture we need to keep in mind is that shown in Fig. 1.

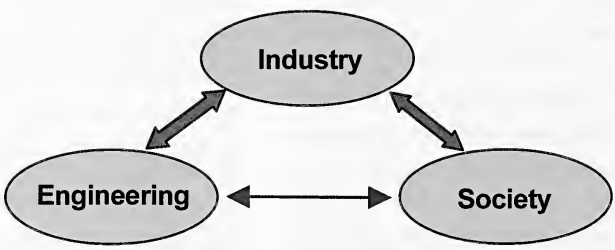

Figure 1: The relationship between engineering and society, with industry as an intermediary.

Only rarely do engineers interact directly with society, free from any considerations of their ties with industry, and the products and services society sees and sometimes associates with engineers are presented by industry. A couple of decades ago Langdon Winner wrote (Winner 1995): "One might suppose that the technical professions offer greater latitude in dealing with the moral and political dimensions of technological choice. Indeed, the codes of engineering societies mention the higher purposes of serving humanity and the public good, while universities often offer special ethics courses for students majoring in science and engineering. As a practical matter, however, the moral autonomy of engineers and other technical professionals is highly circumscribed. The historical evolution of modern engineering has placed most practitioners within business firms and government agencies where loyalty to the ends of the organisation is paramount. During the 1920s and 1930s there were serious attempts to change this pattern, to organise the various fields of engineering as truly independent professions similar to medicine and law, attempts sometimes justified as ways to achieve more responsible control of emerging technologies. These efforts, however, were undermined by the opposition of business interests that worked to establish company loyalty as the engineer's central moral concern (Edwin T. Layton, The Revolt of the Engineers: Social Responsibility and the American Engineering Profession, Cleveland: Case Western Reserve University, 1971, ch.1, 2). Calls for a higher degree of "ethical responsibility" among engineers are still heard in courses in technical universities and in obligatory after-dinner speeches at engineering societies. But pleas of this sort remain largely disingenuous, for there are few legitimate roles or organised settings in which such responsibility can be strongly expressed." This is a major difference to other professions, such as medicine, where there is a direct interface between the profession and society, as illustrated in Fig. 2.

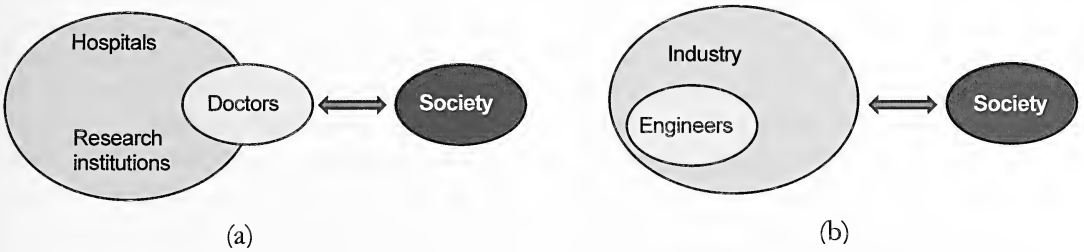

Figure 2: Illustrating the indirect interaction between engineers and society, in (b), as opposed to the direct interaction between doctors and society (their patients), in (a) (from Aslaksen, 2014).

The employment situation in engineering, which is different to that in e.g. medicine and law (although the independence of doctors and lawyers is being eroded, too), is a significant factor in the relationship between engineers and society, as discussed by Aslaksen (2013). The peculiar situation engineers find themselves in is that they are both employers and employees; not like ordinary workers, where their organisations – the unions – are quite distinct from their employers and their organisations. Not only

are the two roles of engineers, employers and employees, evident in industrial companies, but also in the organisations that are supposed to represent the interests of engineers, such as the institutions of engineering. In these organisations, the leadership is usually from the management side of either industry or academia, and there is a potential, and often a real, conflict of interest. The adage "What is good for General Motors is good for the US" is here represented by "What is good for industry is good for engineers".

The current view and utilisation of the engineering profession has become so entrenched that few even entertain the thought that it could be different, and even fewer see that it should be different. If one wants to consider the role of engineers in society, and their social and political responsibilities, one must first look at the current role of engineers in industry. Industry has its own ideology and norms, described by such concepts as profit, value, turnover, growth, return on investment, efficiency, loyalty, and so on, and as long as these norms appear as natural features of society, rather than as something imposed on society, there is little incentive for engineers to question their current role or these norms. It is not a role ordained by Nature; it is a role that has developed and received its current characteristics as part of the capitalist system, and it is a role that can be changed.

Engineers and the Evolution of Society: the processes driving evolution

The number of processes at work in society is very large, and in order to come to grips with them, we might try to introduce some form of taxonomy. As a starting point, we could characterise a society by two groups of entities. In the first group are entities characterising its physical development status, such as housing, public transport, health and education infrastructure, government institutions, and the like. Essentially, this is what Popper called World 1. In the second group are what we might call the society's intellectual content, contained within literature, visual art, music, science, technology, and the like; what Popper called World 3. (Popper's World 2, consisting of mental states, would not be accessible for characterising a society.) Associated with each of these groups are processes that act on the entities; for example, education processes, mining processes, building processes, financial processes, etc. However, for our purpose of investigating what drives changes to society this type of taxonomy is not very useful, as the result of a change to one process will most often result in changes to other processes.

Focusing on the role of technology in changing processes in general, a starting point is to recognise that technology applications can be grouped into two large groups: those resulting in what we might call *unproblematic* changes, and the ones resulting in what we might call *problematic* changes. There is nothing particularly compelling about these names, and there is no sharp boundary between the two groups. In the first group we find those applications of technology that, at the time of their introduction, result in changes that are perceived as being obviously beneficial and not contributing to an area of significant societal concern. Applications of technology in this group include the introduction of electric lighting, the motor vehicle, and such household appliances as the washing machine. The process of acceptance is the one described above; basically the individual's perception of cost-benefit, with some regulatory involvement to ensure public

safety and so on. In the second group are applications that, already at the time of introduction are related to significant societal concerns, such as global warming, biodiversity, civil liberties, and genetic modification. Both because such concerns emerge and change as part of the evolution of society and because the scale of the applications increases to a point where undesirable effects that were initially negligible become significant, applications may move from the first group into the second; a prominent example is coal-fired power generation.

The significance of the two groups to our investigation is that the relationship between technology and society is quite different in each group. In the first group, the information society requires to assess an application is very much directly related to the application; primarily concerned with the performance and cost of the application throughout its life cycle. This is information the engineers would have available as a result of developing the application. In the second group, society requires the additional information about how the application relates to the various societal concerns, and here there are a number of problems. First of all, as in the choice and evaluation of any technology application, one needs to evaluate not only the particular choice of technology in isolation, but also in relation to other possible technologies that could fulfil the same purpose. As these technologies may have different and additional societal concerns associated with them, the evaluation effort is greatly increased. Then, in addition to its intended or primary use, an application may have other uses or features, some of which would potentially change society in an unwanted direction. To what extent should engineers develop and disseminate information about these features? Another problem is that, while the direct interaction of an application with a societal concern may be a matter of straight-forward technical information, such as the amount of carbon dioxide produced, the effect of this on the concern, in this case global warming and climate change, is a different matter, and involves the interface between engineering and science, as well as the distinction between definite data and probabilistic data. This is very evident in the movement to society involvement in technology assessment (or participatory technology assessment, pTA), supported by what is known as post-normal science (Funtowicz and Ravetz 1991, Turnpenny, Jones and Lorenzoni 2011), and exemplified by such organisations as Living Knowledge: The International Science Shop Network (Living Knowledge), the Loka Institute (Loka), the Expert and Citizen Assessment of Science and Technology organisation (ECAST), and World Wide Views (World Wide Views), where there sometimes seems to be confusion between technology, engineering, and science. A further problem is that some concerns have a non-rational basis, such as religious opposition to genetic engineering, and these and other issues make it more difficult to assess what technical information is relevant and appropriate in a given case.

The Nature of Professional Responsibility

That technology and its applications have a significant, and increasing, influence on the evolution of society is generally accepted and well documented, and in Aslaksen (2014) it is argued that the direction in which technology changes society is determined by the collective judgement of the members of society. Furthermore, it was asserted that an important factor in forming that judgement is the information about the technology

available to the members of society, and that the quality of this information will play a major role in determining the direction in which society evolves. This, then, places a significant responsibility on engineers, as the group within society best able to provide this information, and so we have arrived at the questions forming the core of this investigation, such as: How can we define this responsibility? What are the characteristics of the information to be provided? How can engineers discharge this responsibility? How, if at all, is this responsibility currently being addressed? How does it compare with other professional responsibilities?

But before considering this rather special example of professional responsibility, it is useful to take a look at how professional responsibility is treated in the literature. Engineers are not the only professionals faced with problems relating to their responsibilities to society; it is a characteristic of any profession, as the special knowledge of the professional represents a form of power that must be wielded with consideration of its impact on society. In particular, science has many similarities with engineering, and the responsibilities of scientists to society have taken on increasing importance since World War Two. This development, and the issues involved in it, is treated in the book *The responsible scientist*, by John Forge (Forge 2008). It gives a very readable account of the issues involved in defining and understanding the concepts of responsibility, omission, and blame in general, and then reflects this onto the work of scientists, with a number of illustrative examples. Of particular interest in the present context is the distinction between backward-looking and forward-looking responsibility, which Forge attributes to Baier (Baier 1987). Although the distinction can be thought of as arising from the temporal frame of reference, our earlier comments on the nature of the responsibility of engineers for providing information to society show that this responsibility is clearly of the forward-looking kind. Forge discusses the problems associated with assigning forward-looking responsibilities at some length, and illustrates this by the case of the French scientist Joliot.

The Particular Responsibility of Engineers for Providing Information to Society

To put this responsibility into perspective, it might be helpful to compare it with a responsibility in another profession; that of the judiciary in the legal system. In cases before the courts, judges are required to consider the information presented by the stakeholders in the cases, make judgements on the relevance and importance of the information with regard to the specific parts of the law that are applicable to each case, and provide resolutions in the form of sentences or orders. The law, which provides the framework within which cases are brought and which forms the reference for the judges' decisions, is provided by the legislative part of government and does, in principle, reflect the will of the people. The judiciary does not have to make a judgement on whether a particular piece of legislation is good or bad.

The engineers' responsibility is to provide information to society in an unbiased manner and without making any judgement, but as this information is provided prior to society making any judgement regarding the technology, there is no framework to focus or restrict the information processing. Society does define certain requirements on safety, pollution, and the like, but, firstly, these requirements usually refer to existing technology and, secondly, they are focused on preventing any physical harm rather than

making any judgement on the desirability of a particular technology or its influence on society. So, whereas a judge is required to form a judgement about a case, but within the framework of the law, the engineers must not form a judgement regarding the technology application, but must form a judgement regarding the information to be presented to society, without having any prescribed framework to guide them as to what is relevant for this application. In both cases there is a requirement of impartiality, or an absence of any bias in the judgement or the processing of information. In the case of the judiciary, this requirement has been met by making the judiciary independent; in the case of engineers there is, as yet, no corresponding arrangement.

Engineers are no different to anybody else when it comes to basic human traits, such as self-interest and self-delusion, and when engineers that have spent a long time developing a technology and becoming experts in this technology get an opportunity to employ the technology on a project, they are bound to present the application in a favourable light. This is, in principle, no different to the situation in the legal world, where lawyers present their clients' cases in the most favourable light, with the two sides in each case striving for different outcomes; within the law, but without much concern for what is true or best for society. However, there is then an impartial judge who decides the outcome, and there needs to be a corresponding arrangement in engineering if society wants to be assured of receiving quality information about technology. The current approach, in which the proponent of a new application engages a firm of consultants to provide an assessment of costs (incl. risks) and benefits is clearly flawed, as experience with both Environmental Impact Statements (Beder 1995) and some recent failures of major infrastructure projects illustrates (Poljak 2014).

Let us now first consider the nature of the information and what is meant by quality in this case. Seen from a member of society, quality means how appropriate the information is to the task of forming a judgement on whatever application of technology is being put forward. That means that the language in which it is presented must be easily understandable, the depth and level of detail must be appropriate to the education and experience of the person, examples should relate to the person's environment, and it must be easily accessible to that person in a timely manner. It must also describe any relevant alternate solutions, and provide an assessment of their relative merits from a technical standpoint. While these requirements would seem to imply that the information has to be tailored to each individual member of society (and remember, society means all or a group of society, as applicable to the particular application), that would clearly not be practicable, and so there arise the issues of how to package the information, how to subdivide society into groups with relatively homogeneous membership, and how to reach each of these groups in an effective manner. It will require engineers to have a good understanding of how society operates, how groups form, and which groups are relevant to a particular application.

This packaging and targeting of the information will rely heavily on the engineers' judgement of the relevance and importance of items of information to each of the groups, and this brings us to a related issue: the completeness of the information. A problem with much of the current presentation of information about technology is that, without being directly untruthful, the presenters

emphasize those parts of the complete information that are favourable to their purposes, while either suppressing the other parts or implying that they are unimportant or irrelevant. The task facing engineers is to reduce the information provided to what is required for the audience of the information to make a judgement, but in such a manner that it does not bias the judgement. The requirements this task places on the engineers seem to indicate that these engineers would form a distinct group within the profession with regard to both their education and experience, and with a broader perspective than the engineers engaged in the industrial process of developing and applying technology. The need for such a structuring of the profession was raised in a somewhat different context in a recent publication (Aslaksen 2015).

The impact of a new application of technology on society does, as mentioned earlier, depend on numerous factors in addition to those that belong to engineering and about which engineers are best able to provide information. The groups within society with the relevant information about these further factors have the same responsibility as engineers to provide factual, unbiased, and complete information, and it is the combination of all this information that then leads to society's assessment of the application. The process by which such an assessment is reached is highly complex and cannot be usefully defined in closed form. It potentially involves all the human elements of society as a system, but the importance of an element in the assessment and acceptance process varies greatly from element to element and from case to case. It is also a dynamic process which is currently changing towards broader society participation, as already noted, and as a result of the increasing presence of mass media (Petersen, Heinrichs, and Peters 2010). The important point as far as the responsibility of engineers is concerned is that as participants in that process they need to clearly differentiate between when they, as members of the profession, provide information and when they, as members of society, assess the impact of the information provided by both themselves and others.

There is then the question of the scope of the responsibility. Producing the information is one thing, but that work only has an effect if the information is also put to use by society. To what extent does the engineers' responsibility include developing and managing the process of presenting the information to society? And even actively assisting society in understanding and using the information? It would seem that at the very least it would include making society aware of the existence of the information and how to get access to it, which already points to an on-line database in which to deposit the information and from which it can be conveniently extracted, and some form of organisation to maintain it. An early example of technology assessment was provided by the Office of Technology Assessment, an office of the US Congress that operated from 1972 until 1995, and produced more than 750 reports on new technology. These reports are now stored at Princeton University, and available [1]. Today there are numerous organisations dedicated to the relationship between science, technology, and society, such as the Danish Board of Technology (DBT), the Swiss Centre for Technology Assessment (Swiss), the Rathenau Institute (Rathenau), the Institute of Technology Assessment (ITA) of the Austrian Academy of Sciences, and the Norwegian Board of Technology (NBT). However, these organisations are mostly concerned with assessing the impact of a given technology on

[1] http://www.princeton.edu/~ota/ns20/legacy_h.html.

society and only peripherally concerned with assessing the data provided by the technology developers. That is one reason why they are concentrating on the involvement of science and scientists and largely ignoring the involvement of engineers and the role of industry as a driving force within society. The organisation we are considering would be responsible for providing the technical information about technologies and their applications and about what is involved in their realisation: the changes to the industrial structure, to education and training, and, to some extent, to power structures within society.

The creation of such an organisation raises a number of issues. How would it be funded? If it is to be unbiased and impartial, it could not accept funding from any party with an interest in the information and would have to be funded as a statutory body, although it could probably start its life in a less formal guise. To what extent would it be liable for the information provided to society, and how could such a liability be covered? If the information is provided on an "all care and no responsibility" basis, which seems most likely, the credibility will rest solely on the reputation of the person(s) that provide the information. In that case, the organisation is essentially a clearing-house for the information, but it would have to ensure that only reputable engineers with no conflict of interest are accepted as providers.

There are literally hundreds of new applications of technology presented to society every day. Many of these are products and services that are scrutinised by consumer and other organisations that base their assessment on well-established standards (safety, energy efficiency, environmental impact, etc.), and for which no further technical information is required. There therefore has to be a process of selecting those applications on which the public has to (or should) make an assessment based on technical information, and the engineers' organisation would have to be free of any ideological bias in making this selection.

Could any of the existing engineering organisations take on this additional role related to discharging the engineers' responsibility for the evolution of society? There are basically two types of organisations that are relevant: Institutions or Societies of Engineering, and Academies of Engineering. Within each type there are significant variations from country to country, so in order to provide some detail within the space limitation of this paper, the following discussion is specific to Australia. In Australia, The Institution of Engineers (Australia), trading under the name Engineers Australia (EA), is the main organisation representing engineers. However, it does not represent only Professional Engineers, but also Engineering Technologists and Engineering Associates. These three groups are defined briefly as follows:

Professional Engineer: Requires at least the equivalent of the competencies in a four year full time Bachelor's Degree in engineering.

Engineering Associate: Requires at least the equivalent of the competencies in a three year full time Bachelor's Degree in engineering.

Engineering Technologist: Requires at least the equivalent of the competencies in a two year full time Associate Degree in engineering or a two year full time Advanced Diploma in engineering from a university or TAFE (Technical and Further Education) college.

Together, these three groups are called the *engineering team*, and EA considers them all to be members of the engineering profession.

According to the Census of 2011, the engineering team had 322,523 members, of which about 80% participated actively in the labour market. Of this labour force 80%, or about 206,000, were Professional Engineers. Engineers Australia has about 100,000 members, of which 41% are students, 53.5% professional engineers, and 4.5% Technologists and Associates, so that EA represents only about one quarter of the Professional Engineers in Australia.

With regard to providing information to society, EA produces two types of documents: policy statements, and submissions to Government. An example of the former is the one published in 2007, *Climate Change and Energy*, a comprehensive and clearly formulated document, setting out what EA supports and what actions EA believes need to be undertaken. But it is a "passive" document, offering advice and reflecting the fact that EA and the profession have no power to demand or initiate any action. An example of a submission to Government is the recent *Submission to the Senate Economics References Committee into Australia's Innovation System*, published in July 2014. Again, a very well argued submission, but essentially "begging" Government to recognise the role of engineers in innovation.

What these documents demonstrate is a cultural problem, and the questions EA should be asking itself, provide answers to, and find solutions to, include: Why are not a significant proportion of federal and state cabinet ministers engineers? Why do engineering courses not have at least as high ATARs (Australian Tertiary Admission Ranking) as medicine and law? As it stands, and with its watering down of the profession into the engineering team, EA does not appear to be suitable to take on the role of the organisation discussed above.

The Australian Academy of Technological Sciences and Engineering (ATSE) advocates for a future in which technological sciences, engineering and innovation contribute significantly to Australia's social, economic and environmental wellbeing. The Academy is empowered in its mission by some 800 Fellows drawn from industry, academia, research institutes and government, who represent the brightest and the best in technological sciences and engineering in Australia. The Academy provides robust, independent and trusted evidence-based advice on technological issues of national importance. ATSE fosters national and international collaboration and encourages technology transfer for economic, social and environmental benefit. ATSE would, in principle, have the reputation and integrity track record required for our engineering organisation, but there is a big difference between giving high-level policy advice to organisations and providing useful technical information to the general public, and it is doubtful if ATSE would even contemplate such a role.

In the book *The responsible scientist*, introduced earlier (*op. cit.*), Forge goes on to examine how the scientific community is discharging its responsibilities. Many scientific societies, including the Royal Society of London, the Acadèmie des Sciences of Paris, and the American Physical Society, represent science as an activity that is good and worth pursuing in itself, and are dedicated to furthering pure science without any consideration of its impact on society. But there are also a number of other societies whose raison d'être is a concern with wider issues, and he mentions a sample of three. The first is the Federation of American Scientists (FAS), founded in 1945 by some of the members of the Manhattan Project who were concerned about control of the awesome new

technology they had helped create. The second is the Union of Concerned Scientists (UCS), founded in 1969 at MIT, which aims to devise means for turning research applications away from the present emphasis on military technology toward the solution of pressing environmental and social problems. The third is the British-based Scientists for Global Responsibility (SGR), which has similar aims to UCS, but is more explicit in stating what research it believes scientists should and should not be involved in.

All of these organisations have a central point in common, both between themselves and with the organisation we are contemplating: they advocate something regarding what the members of their professions should and/or should not do. But there is a major difference between all those scientific organisations and our organisation: they are concerned with the members' conduct of their professional work, whether they should do certain types of work, and so on, whereas our organisation is concerned with providing society with information about its members', i.e. the engineers, work. We are, in this paper, not concerned with the type of work engineers do and the results of their work; we are solely concerned with ensuring society is in a position to make informed assessments about the resulting products and services.

What this comparison with science is starting to point out is that, while it is important to have an organisational structure to provide an interface to society, there also has to be a societal or legal framework that allows the flow of information to take place. This brings us back to the discussion, in Sec. 2, about engineers being embedded in industry, and how this limits their ability to provide information to society. For engineers to be able to provide complete and unbiased information, society will have to *demand* it, and insist, through the political process, that a corresponding legal framework be put in place. The *right to know* needs to be accepted as an inherent feature of an advanced society. Just as patients have the right to know all they want about their treatments, society should have the right to assess how technology applications will affect it, and the information provided by engineers forms an important part of that process. And from the foregoing discussion of the various existing organisations involved, it seems likely that it will require an act of political will and the involvement of government to create the type of organisation we are advocating.

The three main issues we have identified: the standing of the engineering profession in society, the visibility of the relationship of engineering with technology, and the ability of engineers to provide information to society, are all closely interrelated, and they need to be addressed in a coordinated fashion when assessing the responsibility of engineers to society.

References

Archibald, R.W. and J.P. Newhouse, 1980, *Social Experimentation: Some Whys and Hows*, Rand Report R-2479-HEW, available at www.rand.org/content/dam/rand/pubs/reports/2007/R2479.pdf

Aslaksen, E.W, 2012 *An Engineer's Approach to the Philosophy of Engineering*, Abstracts of fPET-2012, the Forum on Philosophy, Engineering and Technology conference, hosted by the University of the Chinese Academy of Sciences in Beijing, November 2-4, 2012. The full paper to be published as a chapter in the Springer Series on Philosophy of Engineering and Technology.

-, 2013, *The System Concept and Its Application to Engineering*, Springer, sec. B2.1, which refers to works by S.C. Florman and by P. Galloway.

-, 2013a, *The Engineering Paradigm*, Int'l J. of Engineering Studies, vol. 5, No. 2, pp. 129-154.

-, 2014, *The Value of Engineering to Society*, Abstracts of fPET-2014, the Forum on Philosophy, Engineering and Technology conference, hosted by Virginia University of Technology, Blacksburg, VA, 27-29 May 2014. The full paper to be published as a chapter in the Springer Series on Philosophy of Engineering and Technology, under the title *Engineers and the Evolution of Society*.

-, 2015, *Engineers and Society*, submitted to the Australasian Journal of Engineering Education.

Baier, K, 1987, *Moral and Legal Responsibility*, in *Medical Innovation and Bad Outcomes*, M. Siegler (ed.), Health Administration Press, Ann Arbor, p. 103.

Beder, S, 1995, *Ethics, Engineers and Sustainable Development*, 10th Int'l Congress of Logic, Methodology and Philosophy of Science, Florence, Italy.

Callon, M, 1987, *Society in the Making: The Study of Technology as a Tool for Sociological Analysis*, in *The Social Construction of Technological Systems*, Bijker, Hughes, and Pinch (eds.), The MIT Press.

Christensen, S.H, M. Meganck, and B. Delahousse, (eds), 2007, *Philosophy in Engineering*, Academica.

DBT, Danish Board of Technology, www.tekno.dk.

Dessauer, F, *Streit um die Technik*, Verlag Josef Knecht, 1956.

Dorrestijn, S, 2012, *Technical Mediation and Subjectivation: Tracing and Extending Foucault's Philosophy of Technology*, Philos. Technol. 25:221–241.

ECAST, Expert and Citizen Assessment of Science and Technology, http://ecastnetwork.org, accessed on 10.10.2014.

Ellul, J, 1980, *The Technological System*, The Continuum Publishing Corp.

Ferry, L, 2005, *What is the Good Life?*, trans L.G. Cochrane, The University of Chicago Press.

Forge, J, 2008, *The responsible scientist*, University of Pittsburgh Press.

Funtowicz, S.O. and Jerome R. Ravetz, 1991, *A New Scientific Methodology for Global Environmental Issues.* In *Ecological Economics: The Science and Management of Sustainability.* Robert Costanza, ed. New York: Columbia University Press: 137–152. For a more recent overview, see *Post-Normal Science: environmental Policy under Conditions of Complexity*, available at http://www.nusap.net/sections.php/op=viewarticle&article=13, accessed 08.10.2014.

Hausman, J.A. and D.A. Wise, 1980, *Introduction to "Social Experimentation",* ch. 1 in *Social Experimentation*, Hauseman and Wise (eds.), Uni. of Chicago Press.

Hector, D.C.A, 2012, *Towards a Modern Engineering Ethical Framework: The Foundations of Western Engineering Ethics*, Abstracts of fPET-2012, the Forum on Philosophy, Engineering and Technology conference, hosted by the University of the Chinese Academy of Sciences in Beijing, November 2-4, 2012. The full paper to be published as a chapter in the Springer Series on Philosophy of Engineering and Technology.

Heidegger, M, 1977, *The Question Concerning Technology*, available online at http://simondon.ocular-witness.com/wp-content/uploads/2008/05/question_concerning_technology.pdf, accessed 10.08.2014.

Hughes, T.P, 1987, *The Evolution of Large Technological Systems*, in *The Social Construction of Technological Systems*, Bijker, Hughes, and Pinch (eds.), The MIT Press.

ITA, Institute of Technology Assessment of the Austrian Academy of Sciences, www.oeaw.ac.at/ita.

Li, Bo-cong, 2010, *The Rise of Philosophy of Engineering in the East and the West*, in *Philosophy and Engineering*, I. van de Poel and D.E. Goldberg (eds), Springer.

Loka Institute, www.loka.org, accessed on 08.10.2014.

LSJ (1940), *Dictionary of Ancient Greek*, Liddell, Scott, Jones, Clarendon Press, also available online at www.perseus.tufts.edu, accessed 12.03.2013.

Knoedler, J. and A. Mayhew, 1994, *The Engineers and Standardization*, Business and Economic History, vol. 23, no. 1.

Living Knowledge, www.livingknowledge.org, accessed on 08.10.2014.

MacKenzie, D. and J. Wajcman (eds.), 1999, *The social shaping of technology*, Open University Press, 2nd ed.

Marx, L, *The Idea of "Technology" and Postmodern Pessimism*, in *Does Technology Drive History?*, M.R. Smith and L. Marx (eds.), The MIT Press (1994).

NBT, Norwegian board of Technology, http://teknologiradet.no, accessed 06.10.2014.

Newberry, B, 2007, *Are engineers instrumentalists?*, Technology in Society 29.

Noble, D.F, 1977, *America by design: science, technology, and the rise of corporate capitalism*, Alfred Knopf.

Petersen, I, H. Heinrichs, and H.P. Peters, *Mass-Mediated Expertise as Informal Policy Advice*, Science, Technology, & Human Values, vo. 35, no. 6, pp. 865-887.

Rathenau Institute, www.rathenau.nl, accessed 06.10.2014.

Swiss Centre for Technology Assessment, https://www.ta-swiss.ch, accessed 06.10.2014.

Turnpenny, J, M. Jones, and I. Lorenzoni, 2011, *Where Now for Post-Normal Science?: A Critical Review of its Development, Definitions, and Uses*, Science, Technology, & Human Values, vol. 36, no. 3, pp 287-306.

Unger, S.H, 1994, *Controlling Technology: Ethics and the Responsible Engineer*, John Wiley & Sons, 2nd ed.

Vann, W.P, 1997, Bibliography on the subject of ethics in engineering, available at http://ethics.tamu.edu/References.aspx, accessed 10.08.2014.

Veblen, T, 1921, *The Captains of Finance and the Engineers*, p.61, in *The Engineers and the Price System*, B.W. Huebsch, Inc. New York.

Winner, L, 1995, *Citizen Virtues in a Technological Order*, Ch. 4 in *Technology and the Politics of Knowledge*, A. Feenberg and A. Hannay (eds.), Indiana University Press.

World Wide Views, www.wwviews.org, accessed 08.10.2014.

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3D Bridge Microdosimeter: Charge Collection Study and Application to RBE Studies in ^{12}C Radiation Therapy

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Abstract

Radiotherapy using a heavy ion beam such as the Carbon-ion has an advantage for the treatment of deep-seated tumours over conventional radiotherapy with X-rays due to an enhanced dose deposition in the Bragg peak (BP) at the end of the ion range. The highest dose can be deposited in the tumour with much lower doses to the surrounding healthy tissue. The Relative Biological Effectiveness (RBE) of a carbon-ion radiotherapy beam greatly depends on the depth of the target volume in the body and the nuclear fragmentation process that increases close to the BP or spread out BP (SOBP) as well as neutrons. It is important to understand the RBE of the heavy ions in hadron therapy applications in order to deliver the correct dose.

Microdosimetry is an extremely useful technique, used for RBE studies in unknown mixed radiation fields typical of hadron therapy. Conventional detectors for microdosimetry consist of tissue equivalent proportional counters (TEPC) which have the advantages of a spherical sensitive volume and tissue equivalency through use of a tissue equivalent gas. However, TEPC has several limitations such as high voltage operation, large size of assembly, which reduces spatial resolution and introduces wall effects, and an inability to simulate multiple cells.

A new silicon microdosimeter with 3D sensitive volumes (SVs) has been proposed to overcome the shortcomings of the conventional TEPC. The new microdosimeter is called a "bridge" microdosimeter as it has thin Si bridges between the SVs to support the Al tracks over the SVs. The charge collection study of the new device and its application for RBE determination in ^{12}C radiation therapy at the Heavy Ion Medical Accelerator in Chiba (HIMAC), Japan, is presented.

This work presented the first RBE_{10} derivation in a ^{12}C ion therapeutic beam using a high spatial resolution silicon microdosimeter and demonstrated a simple and fast method for Quality Assurance in charged particle therapy.

Introduction

According to the NSW Cancer Registry Statistical Reporting, 1% of the population per annum is diagnosed with cancer. Approximately one third of Australians are expected to develop cancer during their lifetime with about two thirds of cancer in people aged over 65. More than 50% of all patients with localised malignant tumours are being treated with radiation (Schardt, 2010).

Conventional X-ray radiotherapy is used for treatment of many types of tumour but it has limitations as it also irradiates normal tissues surrounding the tumours especially when tumours are located in proximity to critical organs or in paediatric treatments.

Particle therapy is advantageous for the treatment of deep-seated tumours as the highest dose can be deposited in the tumour with much lower doses to the surrounding healthy tissue. The energy deposition mechanisms of ions in matter are different to photons and are dominated by electronic collisions for the relevant energies of primary ions described by the Bethe-Bloch formula (Bethe, 1930, Bloch, 1933). The nuclear reactions contribute substantially to the ion dose via nuclear fragments and neutron production. Fig. 1 shows a comparison of the depth dose distribution produced by MV photons and different energies of the ^{12}C ions.

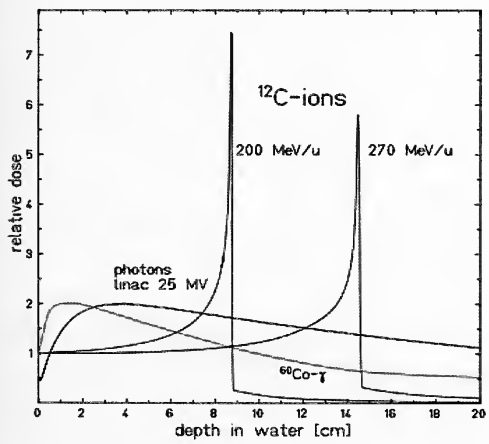

Figure 1. Depth-dose profiles of ^{60}Co γ radiation, megavolt photons, and ^{12}C ions in water (Schardt, 2010). The fragmentation tail is clearly observed downstream of the ^{12}C Bragg peak.

In order to accurately predict radiobiological effects in humans due to the radiation field of a typical cancer treatment with ions, it is important to understand how the Linear Energy Transfer (LET) of primary and secondary ions varies with depth as well as with its RBE.

Microdosimetry is a method of measuring the microscopic pattern of ionizing energy deposition in a micron-sized sensitive volume (SV) of similar dimensions to biological cells (Rossi, 1996). This technique is extremely useful for understanding the radiobiological properties of unknown mixed radiation fields typical of space and aviation, as well as in hadron therapy. The microdosimetric quantity used to describe the energy deposition in such a SV along a particle track is called the lineal energy deposition (y).

$$y = \frac{E}{\langle l \rangle} \quad (1)$$

where E is the ionizing energy deposited in a micron-sized SV with an average chord length $\langle l \rangle$ from a single event. The spectrum of stochastic events $f(y)$ for all primary and secondary particles generated during an exposure of tissue to ionizing radiation can be derived from the spectrum of energy deposition events. The partial fraction of dose deposited by the charged particles within lineal energy interval $(y, y+dy)$ is $d(y)$ and is given by:

$$d(y) = \frac{yf(y)}{\overline{y_F}}, \quad (2)$$

where $\overline{y_F} = \int_0^\infty yf(y)dy$ is the frequency mean lineal energy and $d(y)$ vs. y is a microdosimetric spectra usually presented in a log scale as $yd(y)$ vs. $\log(y)$. The major microdosimetric parameter relevant to RBE and derived from microdosimetric spectra is $\overline{y_D} = \int_0^\infty yd(y)dy$, where $\overline{y_D}$ is the dose mean lineal energy. Using the microdosimetric spectra and the

microdosimetric kinetic model (MKM; Kase, 2011) the RBE of the ^{12}C ion beam can be derived.

The Centre for Medical Radiation Physics (CMRP) at the University of Wollongong has initiated the concept of silicon microdosimetry to replace the current microdosimetry gold standard, the TEPC. Compared to the TEPC, the CMRP silicon microdosimeters are advantageous due to being a solid-state detector with no gas-flow ensemble, having very low operating voltages less than 10V, extremely high spatial resolution of up to 10μm and a high degree of portability and ability to simulate multiple cells (Bradley 1998, 2000). Three generations of silicon on insulator (SOI) microdosimeters have been developed, fabricated and investigated (Bradley 2000, Cornellius 2003, Ziebell 2008, Livingstone 2012). Based on previous research and development at CMRP, the feasibility of the silicon microdosimetry concept has been proven. Despite this success, a number of limitations have been observed in previous designs of the SOI microdosimeter. These limitations include non-uniformity of charge collection in the SV and diffuse charge collection outside of the SV (Ziebell, 2008). In order to overcome these drawbacks, we have proposed further steps to optimize the SOI microdosimeters with the development of freestanding 3D SVs, the so called "mushroom" microdosimeter, using 3D detector technology (Tran, 2014). A Geant4 simulation study has been carried out to optimize the design and investigate the response of the "mushroom" microdosimeter in aviation neutron fields. These results have given confidence to the new microdosimeter design (Tran, 2014). However, the manufacturing process for fabricating free-standing 3D SVs on a silicon substrate is complex. As an intermediate step towards free-standing 3D SVs, an SOI microdosimeter with 3D SVs was produced by etching the silicon surrounding the SVs whilst leaving thin silicon "bridges" between the SVs to support the aluminium tracks electrically connecting SVs. The new microdosimeter is called the "bridge" microdosimeter as it has thin Si bridges connecting the SVs. The charge collection study of the new device and its application for RBE determination in ^{12}C radiation therapy is presented.

Material and Method

Design of the 3D bridge microdosimeter

The newly developed bridge microdosimeter has a large sensitive area of 4.1 x 3.6mm^2 designed for use in low dose rate environments such as those in aviation and space. The device is segmented into three sections in order to reduce the noise by minimizing the capacitance and dark current of each segment (Fig. 2a). Fig 2b shows a SEM image of an array of SVs of the 3D bridge microdosimeter where the surrounding silicon was fully etched down to 10μm depth using the deep reactive ion etching (DRIE) technique which produced a straight parallelepiped SV shape. This new technology provided well-defined geometry of micron-sized 3D SVs.

The microdosimeter is based on an array of 4248 planar 30x30x10μm cubic SVs fabricated on a high resistivity n-SOI active layer of thickness 10μm and a low resistivity supporting wafer. Layers of phosphorus silicate glass (PSG) and SiO_2 were deposited on top of the device. Each SV was fabricated using ion implantation to produce the square p-n junction structure (Fig. 2c). The even and odd rows of SVs are read out independently to avoid events in adjacent sensitive volumes being read as a single event in the case of oblique charged particle tracks.

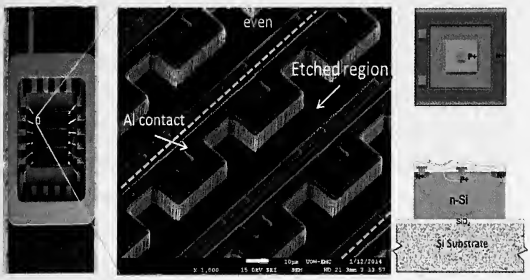

Figure 2: a) A bridge microdosimeter mounted on a Dual In Line (DIL) package, b) a SEM image of arrays of SVs, c) A top view and a cross-section of the SV of microdosimeter.

Ion Beam Induced Charge Collection (IBICC) Technique

The charge collection efficiency for the 3D mesa bridge microdosimeter was investigated using the Ion Beam Induced Charge Collection (IBICC) technique at the Australian National Tandem for Applied Research (ANTARES) heavy ion microprobe at Australian Nuclear Science and Technology Organisation (ANSTO) (Siegel, 1999). A monoenergetic beam of ions focused to a diameter of approximately 1μm was raster scanned over the surface of the microdosimeter. A 5.5MeV He^{2+} ion microbeam was used in this study. The IBICC signal corresponding to the beam position (X,Y) as well as the charge collection E for each event was processed into an event-by-event list mode file. Median energy maps showing the charge collection characteristics of the device were then created. Energy calibration of the spectroscopy chain was performed using a pulse generator which was calibrated with a 300μm thick planar silicon PIN diode with 100% Charge Collection Efficiency (CCE) exposed to the ion sources used in the IBICC experiment.

Experiment at ^{12}C ion therapy facility – Heavy Ion Medical Accelerator in Chiba (HIMAC), Japan

The 3D bridge microdosimeter was placed in various positions along the central axis of the Spread Out Bragg Peak (SOBP) of a 290MeV/u ^{12}C ion beam at the Heavy Ion Medical Accelerator in Chiba (HIMAC), Japan. A modular polymethyl methacrylate (PMMA) phantom was used to adjust the position of the Bragg peak relative to the device.

The cell survival irradiated with an absorbed dose of ionizing radiation D is described by the Linear Quadratic Model (LQM) as:

$$S = \exp(-\alpha D - \beta D^2), \tag{3}$$

The Relative Biological Effectiveness (RBE_{10}) of the ^{12}C ion beam is defined as the ratio of the absorbed dose required to achieve 10% cell survival using X-rays to that required when using the radiation of interest.

The modified MK model (Kase, 2011) relates the microdosimetric parameter – dose-mean lineal energy $\overline{y_D}$ to the LQM parameter α, for a particular radiation field. Using the LQM of the cell survival response to radiation, the RBE_{10} can be expressed as:

$$RBE_{10} = \frac{2\beta D_{10,R}}{\sqrt{\alpha^2 - 4\beta \ln(0.1)} - \alpha}, \tag{4}$$

where α, β are tissue radio-sensitivity coefficients (α in units of Gy^{-1} and β in units of Gy^{-2}). $D_{10,R}$ = 5.0Gy is the 10% survival dose for human salivary gland (HSG) tumour cells using 200 kVp X-rays.

$$\alpha = \alpha_0 + \frac{\beta}{\rho \pi r_d^2} y^*, \tag{5}$$

where α_0=0.13Gy^{-1} is a constant that represents the initial slope of the survival fraction curve in the limit of zero LET,

β=0.05Gy^{-2} is a constant independent of LET, ρ=1 g/cm^3 is the density of tissue and r_d=0.42μm is the radius of a sub-cellular domain in the MK model.

$$y^* = \frac{y_0^2 \int_0^\infty (1-\exp(-y^2/y_0^2)) f(y) dy}{\int_0^\infty y f(y) dy} \quad (6)$$

where y^* is a restricted dose-mean lineal energy which is taking into account the cell overkilling effect for lineal energy larger than y_0 = 150keV/μm. The same parameter y_0 was used at HIMAC in the experiments with the TEPC. The conversion factor of 0.63 was used to convert the lineal energy deposition in silicon to tissue equivalent material (Bradley, 1998).

Results and Discussions

Charge Collection Studies

The response of the 3D bridge microdosimeter was investigated using 5.5 MeV high LET He^{2+} ions. Fig. 3 shows MCA (Multi-channel analyser) spectra and median energy maps obtained from odd and even arrays of the 3D bridge microdosimeter.

The microbeam was scanned across the microdosimeter using a scanning area of 0.3mm x 0.3mm. The microdosimeter was biased at –10 V. At –10 V the peak of the deposited energy distribution in the 10μm bridge microdosimeter is approximately 1350keV which is close to the expected value of 1480keV from 5.5MeV He^{2+} in 10μm silicon and 1μm SiO_2 over-layer, calculated by SRIM 2008 (Ziegler, 2008). Compared to previous generations of SOI microdosimeters, low energy events no longer exist in the 3D bridge microdosimeter because of excellent etching technique, removing silicon surrounding the SVs.

Fig. 3b shows full charge collection in the SVs and slightly less than 100% charge collection efficiency in the bridge region due to diffused charge collection.

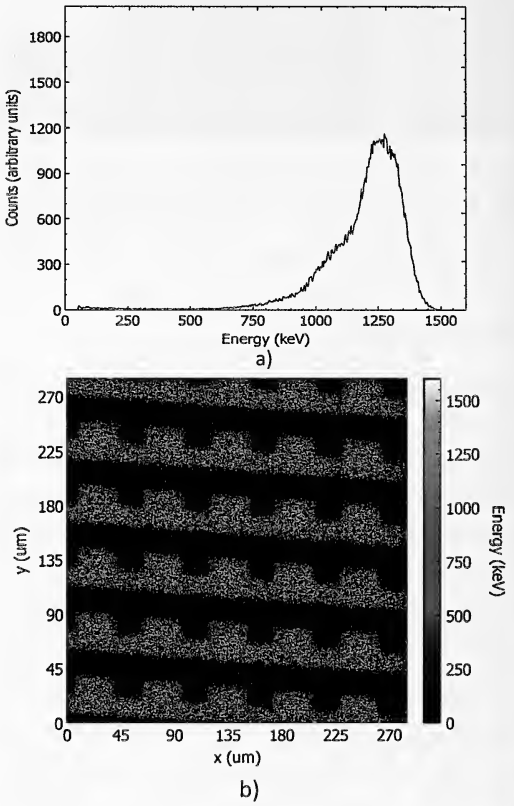

Figure 3. Response of 3D bridge microdosimeter to 5.5MeV He^{2+} ions biased at –10V. (a) Energy spectrum and (b) median energy 2D map.

Experiment at ^{12}C heavy ion facility in Chiba, Japan

Derived RBE_{10} values based on the MK model and 3D bridge microdosimetric spectra in response to 290MeV/u carbon-ions is presented in Fig. 4. The RBE_{10} values match very well with those obtained from the TEPC measurements. Due to the high spatial resolution of the microdosimeter, a more detailed RBE_{10} distribution was obtained at the end of the SOBP compared to the TEPC. The maximum derived RBE_{10} found using

the bridge microdosimeter was 2.56 that is higher than the one of 2.35 obtained with the TEPC due to high spatial resolution of the SOI microdosimeter which was able to measure at the end of the SOBP with 0.5 mm resolution. In general, the obtained RBE_{10} values were found to be in good agreement with values obtained using a TEPC, with an exception immediately downstream of the SOBP in the fragmentation region. This is due to two facts: i) The TEPC measurements being carried out in water which lacks the C atoms in PMMA; ii) the spatial resolution of the TEPC is not good enough to measure separately the distal part of the SOBP. This region is determined by low energy ^{12}C ions with high LET and the fragmentation region with the LET dropping fast below 150keV/μm. This leads to an effective increase in RBE_{10} where the chosen parameter of the overkilling effect is y_0 = 150 keV/μm (equation 6). It should be noted that the bridge microdosimeter measurements were done in a PMMA phantom while the TEPC measurements were carried out in water, hence range scaling has been used to match the results.

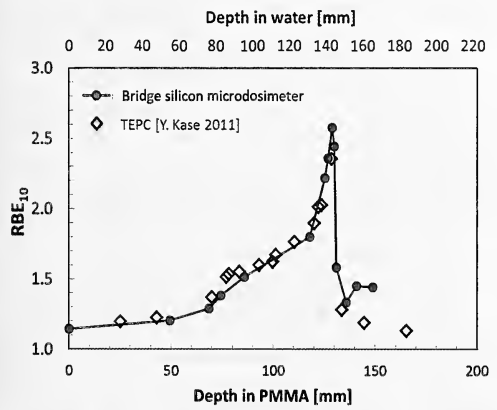

Figure 4. Derived RBE_{10} along the central axis of the SOBP of ^{12}C ion beam, obtained by SOI bridge microdosimeter and TEPC at NIRS.

Conclusions

The CMRP 3D bridge microdosimeter was investigated in detail using 5.5 MeV He^{2+} microbeams. The etching of silicon surrounding the SVs was shown to remove all low energy artefacts in comparison with planar SOI microdosimeters.

This work presented the first RBE_{10} derivation in a ^{12}C ion therapeutic beam using a high spatial resolution SOI microdosimeter. The obtained RBE_{10} values were found to be in good agreement with values obtained using a TEPC, with an exception at the distal part of the SOBP. This is due to TEPC measurements being carried out in water which lacks the C atoms in PMMA and a lack of high spatial resolution in the TEPC.

The development of an extremely high spatial resolution SOI microdosimeter is very important for a better understanding of radiobiological dosing in heavy ion therapy as well as aspects of the physics of ions such as the fragmentation process and deposition of energy at the end of the BP.

This current bridge SOI microdosimeter is an intermediate step towards a fully 3D microdosimeter with a free-standing 3D SVs microdosimeter.

Future Work

The proposed 3D SVs microdosimeter ("mushroom" microdosimeter) is currently being fabricated at SINTEF MiNaLab. The design of this 3D microdosimeter was proposed by CMRP, and it has 3D cylindrical SVs to provide a well-defined sensitive region. Fig 5 shows the proposed fully 3D microdosimeter. It is fabricated on SOI material with a buried oxide layer that isolates the microdosimeter's SV from the support wafer. An array of n^+ electrodes and surrounding ring p^+ electrodes are produced using DRIE, followed by polysilicon

deposition and doping. The proposed microdosimeter is also important for characterization of RBE in neutron fields of aviation, space and accelerator radiation environments for radiation protection. To improve the tissue equivalency of the new microdosimeter, the support handle wafer will be removed and filled with a tissue equivalent material such as polymethyl methacrylate (PMMA).

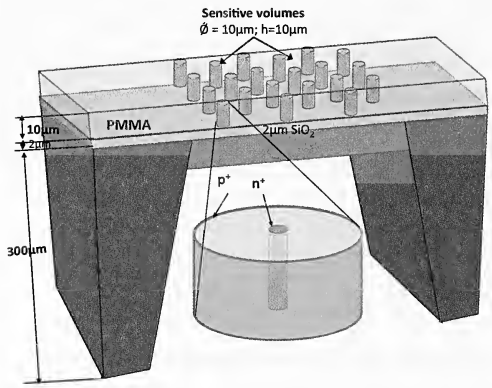

Figure 5. Proposed design of a fully 3D "mushroom" microdosimeter.

Future work will also be focused on comparing the experimental response of the 3D bridge and 3D mushroom microdosimeters in a ^{12}C ion therapy beam with Geant4 simulations.

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References

Bethe, H. (1930) "Theory of the Passage of Fast Corpuscular Rays Through Matter" Ann. Phys., 397, 325-400.

Bloch F. (1933) "Bremsvermogen von Atomen mit mehreren Elektronen", Z. Phys., 81, 363-376.

Bradley, P.D., Rosenfeld, A.B., Lee, K.K. Jamieson, D.N., Heiser, G. and Satoh, S. (1998) "Charge collection and radiation hardness of a SOI microdosimeter for medical and space applications," *IEEE Trans. Nucl. Sci.*, vol. 45, no. 6, pp. 2700–2010.

Bradley, P.D. and Rosenfeld, A.B. (1998) "Tissue equivalence correction for silicon microdosimetry detectors in boron neutron capture therapy," *Medical Physics*, vol. 25, no. 11, pp. 2220–2225.

Bradley, P. (2000) "The development of a novel silicon microdosimeter for High LET radiation therapy," Ph.D. Thesis, University of Wollongong, Wollongong, Australia.

Cornelius, I.M., Rosenfeld, A.B., Seigele, R., and Cohen, D. (2003) "LET dependence of the charge collection efficiency of silicon microdosimeters," *IEEE Trans. Nucl. Sci.*, vol. 50, no. 6, pp. 2373–2379.

ICRU, (1983) "Microdosimetry," *ICRU Report 36*.

Kase, Y., Kanai, T. Sakama, M., Tameshige, Y., Himukai, T., Nose, H., Matsufuji, N. (2011) "Microdosimetric Approach to NIRS-defined Biological Dose Measurement for Carbon-ion Treatment

Beam," *Journal of Radiation Research*; ISSN: 0449-3060; vol. 52, no.1, pp. 59–68.

Lim, W.H., Ziebell, A.L., Cornelius, I., Reinhard, M.I., Prokopovich, D.A., Dzurak, A.S., Rosenfeld, A.B. (2009) "Cylindrical silicon–on-insulator microdosimeter: Design, fabrication and TCAD modelling," *IEEE Trans. Nucl. Sci.*, vol. 56, no. 2, pp. 424–428.

Livingstone, J., Prokopovich, D.A., Lerch, M. L.F., Reinhard, M.I., Petasecca, M. and Rosenfeld, A.B. (2012) "Large Area Silicon Microdosimeter for Dosimetry in High LET Space Radiation Fields: Charge Collection Study," *IEEE Trans. Nucl. Sci.,* vol. 59, no. 6, pp. 3126–3132.

NSW Cancer Registry Statistical Reporting, www.statistics.cancerinstitute.org.au

Rossi and Zaider M. (1996) "Microdosimetry and its Applications," London: *Springer.*

Schardt, D., Elsässer, T., Schulz-Ertner, D. (2010) "Heavy-ion tumor therapy: Physical and radiobiological benefits" Reviews of Modern Physics, vol. 82, Issue 1, pp. 383-425.

Siegele, R., Cohen, D., and Dytlewski, N. (1999) "The ANSTO high energy heavy ion microprobe," *Nucl. Instrum. Meth. Phys. Res. B*, vol. 158, pp. 31–38.

Tran, L.T., Prokopovich, D.A., Petasecca, M., Lerch, M.L.F., Kok, A., Summanwar, A., Da Via, C., Reinhard, M.I., Schjølberg-Henriksen, K. and Rosenfeld, A.B. (2014) "3D Radiation Detectors: Charge Collection Characterisation and Applicability of Technology for Microdosimetry," *IEEE Trans. Nucl. Sci.,* vol. 61, no 4, pp. 1537–1543.

Tran, L.T., Guatelli, S. Prokopovich, D.A., Petasecca, M., Lerch, Reinhard, M. ., Ziegler, J.F.M. and Rosenfeld A.B. (2014) "A Novel Silicon Microdosimeter using 3D Sensitive Volume: Modelling the Response in Neutron Field Typical of Aviation," *IEEE Trans. Nucl. Sci.,* vol. 61, no. 4, pp. 1552–1557.

Ziebell, A.L., Lim, W.H., Reinhard, M.I., Cornelius, I., Prokopovich, D., Siegele, A., R., Dzurak, A.S. and Rosenfeld, A.B. (2008) "A cylindrical silicon-on-insulator microdosimeter: Charge collection characteristics," *IEEE Trans. Nucl. Sci.,* vol. 55, no. 6, pp. 3414–3420.

Ziegler, J.F., Biersack, J.P. and Ziegler, M.D. (2008) "SRIM – The Stopping and Range of Ions in Matter", *Ion Implantation Press*, Available: http://www.srim.org.

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Unlocking Amniote Live Birth: the 'Other' Mammalian Model

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Abstract

Amniotes (birds, reptiles and mammals) exhibit a remarkable range of reproductive strategies. The transition from oviparity (egg-laying) to viviparity (live birth) has occurred independently multiple times in squamate reptiles (snakes and lizards) and once in therian mammals (placental mammals and marsupials) and requires many changes to the uterus to allow the embryo to develop inside the mother. An important step in this transition is the evolution of a placenta. Formation of a placenta in early pregnancy requires substantial remodelling of the surface of the uterus, termed the plasma membrane transformation. Similar cellular changes occur in both placental mammals and live-bearing squamate reptiles which suggest this phenomenon plays an important role in the evolution of amniote viviparity.

Marsupials are ideally placed to test theories of the generality and importance of the plasma membrane transformation of the uterus. Similar morphological changes also occur in a marsupial species (*Sminthopsis crassicaudata*; Dasyuridae), suggesting these changes are ubiquitous in amniote pregnancy, but remodelling appears to be underpinned by different molecular changes in each group. This study demonstrates that not all uterine changes are common across vertebrate lineages. Thus, the transition from egg-laying to live birth may involve flexible molecular recruitment as common molecules do not play the same roles in pregnancy in different live bearing groups. This study highlights the necessity of including marsupials as a separate mammalian group in comparative studies, and the valuable and novel contribution marsupials can make to evolutionary theories.

Introduction

The transition from egg-laying (oviparity) to live birth (viviparity) has occurred many times within the vertebrates and produced a remarkable diversity of live-bearing species (Blackburn and Flemming, 2009): from mammals, to live-bearing skinks and snakes, and even to male pregnancy in seahorses (Whittington et al., in review). This life history transition involves many complex steps, including a reduction of the eggshell, and development of mechanisms to fulfill the embryo's gas exchange and waste removal requirements during development in the uterus. Repeated evolution of live birth within the vertebrate clade Amniota (birds, reptiles and mammals; Figure 1) has resulted in major differences in gestation length, and the amount of nutrients provided to the embryo during pregnancy. Despite these differences, viviparous members of this clade overcome the novel problems of viviparity in common ways.

The best example of a common strategy is the co-option of the characteristic extra-embryonic membranes of this group, which in egg-laying taxa meet an embryo's needs within an eggshell, to form a placenta in early pregnancy. The placenta is a complex organ that forms from intimate contact between the embryo and the cells of the uterus as the embryo implants, and enables exchange of gases, wastes and, to varying extents, nutrients, to occur between the mother and the embryo within the uterus (Ramirez-Pinilla et al., 2012).

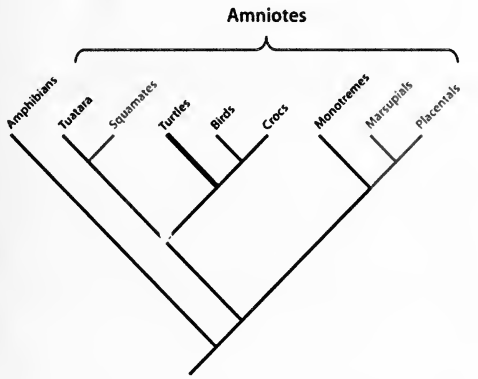

Figure 1: Phylogeny of the amniotes. Blue branches indicate lineages which contain viviparous members.

Placentation requires significant remodelling of the epithelial cells lining the uterus in early pregnancy to enable intimate contact between the uterus and the embryo. Without remodelling, the uterus will not become receptive to implantation by the blastocyst, and pregnancy will fail (Kaneko et al., 2008; Murphy, 2004; Murphy et al., 2000; Orchard and Murphy, 2002; Zhang et al., 2013). Hence, the cell changes that occur during this period, termed the plasma membrane transformation (Figure 2), are critical to determining the success of the pregnancy (Murphy, 2000; 2004; Murphy et al., 2000).

Common Uterine Changes

Recent studies of pregnancy in live-bearing lizards have identified changes that are remarkably similar to those changes involved in preparation for mammalian pregnancy (Biazik et al., 2007; Murphy et al., 2000). In both lizards and placental mammals, uterine cells flatten, and lose microvilli from their apical surfaces (Figure 2), creating a smooth, flat surface to which the embryo can adhere (Murphy, 2004; Murphy et al., 2000; Orchard and Murphy, 2002).

Junctions in the lateral membrane undergo distinct structural changes (Murphy, 2000; Murphy et al., 2000; Orchard and Murphy, 2002). Tight junctions, which regulate fluid transport across the uterine lining, are modified to block unregulated solute movement into the uterus (Biazik et al., 2007; Orchard and Murphy, 2002). This modification enables precise control of the fluid environment surrounding the embryo during implantation and development (Murphy, 2000; Murphy et al., 1982).

The number of desmosomes, or attachment points between epithelial cells, decreases at implantation as the remodelled cells become more labile (Biazik et al., 2010). Thus, the apical and lateral morphological changes shared by squamate reptiles and placental mammals influence both the structural and chemical environment to which the embryo is exposed in the uterus.

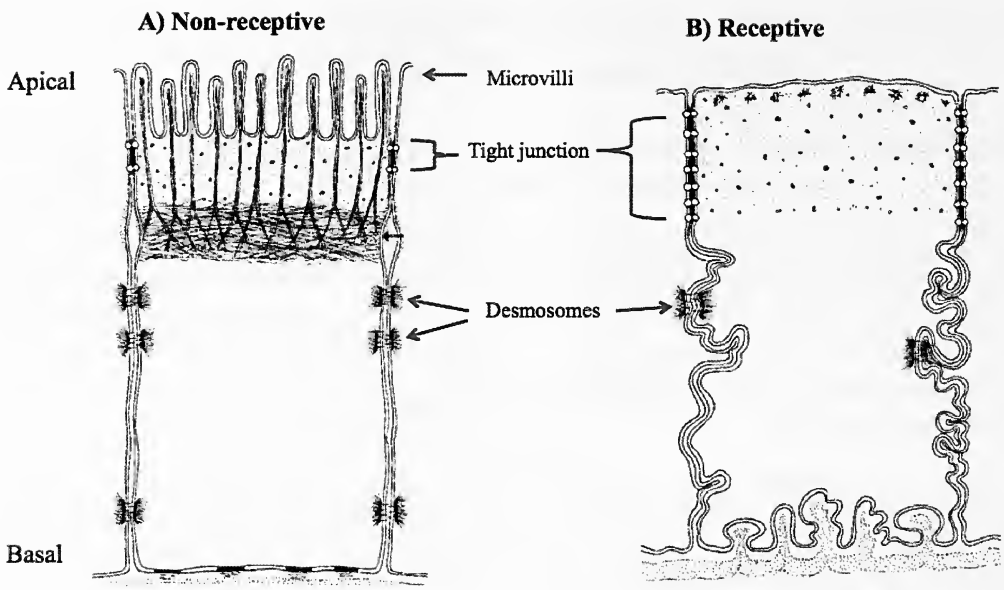

Figure 2: Remodelling of the uterine lining during pregnancy. A) Non-receptive cells have long apical microvilli, short lateral tight junctions and numerous lateral desmosomes. B) Receptive cells have undergone a 'plasma membrane transformation' (PMT) and are remodelled. Apical microvilli are replaced by a smooth flat surface. Laterally, tight junctions extend further down the membrane and the number of desmosomes is reduced. Receptive cells are ready to receive the embryo at implantation (adapted from Murphy, 2000).

Molecular Changes

A common beginning to pregnancy in diverse live-bearing groups suggests that this suite of morphological changes is essential for receptivity and successful pregnancy in live-bearing species. Remarkably, these apical and lateral changes occur whether the embryo breaches the uterine lining and invades maternal tissue, even maternal blood vessels, as it implants (haemochorial placentation), or whether the embryo simply adheres to the uterine lining and does not invade (epitheliochorial placentation; Biazik et al., 2010; Wooding and Flint, 1994). Repeated evolution of morphological changes in diverse groups suggests that the plasma membrane transformation is a fundamental characteristic of live birth in the amniotes (Murphy et al., 2000; Thompson et al., 2002).

While morphological changes are common between viviparous groups, the molecular changes that underpin them are much more variable. For example, modification of tight junctions involves the molecule claudin-5 in both placental mammals and squamate reptiles (Biazik et al., 2008). Occludin, another key tight junction molecule, is also involved in this process in placental mammals, but not all viviparous skink lineages (Biazik et al., 2007). Differences in patterns of key molecules involved in pregnancy suggest that the roles of these molecules differ between lineages (Biazik et al., 2007), and that the common morphological changes shared by different viviparous groups are underpinned by

flexible, or variable, molecular mechanisms (Brandley et al., 2012).

The 'Other' Mammals

Marsupials, while part of the same viviparous lineage as placental mammals, are a highly unusual mammalian group which has been distinct for at least 125 million years (Graves, 1996). The unique features of pregnancy in this group mean that marsupials provide an important and novel perspective to studies of amniote viviparity (Graves and Westerman, 2002; Shaw and Renfree, 2006). A short gestation period (as short as 12 days in *Sminthopsis. crassicaudata*), followed by an extended period of lactation in the pouch (Carter, 2008; McAllan, 2011), during which most organ growth and differentiation of the young occurs (Freyer and Renfree, 2009; McAllan, 2011; Renfree, 2010; Shaw and Renfree, 2006). The marsupial embryo is surrounded by a shell for most of pregnancy and 'hatches' in the uterus several days before birth. As a result, implantation and formation of a choriovitelline placenta do not occur until late in pregnancy, and in some cases, the placenta functions for only a few days (Roberts and Breed, 1994).

Despite the unique features of marsupial pregnancy, a plasma membrane transformation occurs in the marsupial species *Sminthopsis crassicaudata* (the fat-tailed dunnart; Laird et al., 2014), demonstrating that uterine changes are a ubiquitous and essential requirement of amniote pregnancy. The next step is to identify the molecular changes that underpin the plasma membrane transformation in marsupial pregnancy, and to compare the molecular mechanisms with those of other live-bearing mammals. In this way, marsupials provide an ideal model system to test the generality of uterine changes in mammalian pregnancy and to identify their importance in the evolution of live birth in this group.

Basal membrane changes in *Sminthopsis crassicaudata*

We conducted a study of the molecules involved in changes in the basal plasma membrane of uterine cells during pregnancy in a marsupial (*Sminthopsis crassicaudata*; Dasyuridae). Changes in the basal plasma membrane, particularly molecular changes, are potentially the most interesting and informative as they are directly involved in the cellular dynamics of implantation. In the rat, implantation involves sloughing of regions of the uterine lining to facilitate invasion of the embryo into the underlying maternal blood vessels (Enders and Schlafke 1967). Sloughing requires disassembly of protein complexes (focal adhesions) that anchor the uterine lining to the underlying uterine tissue. This process involves loss of focal adhesion molecules, including talin and paxillin, from the basal region of cells before the embryo can implant (Kaneko et al., 2008; 2009).

Highly invasive implantation and sloughing are rare among viviparous species. Most eutherian embryos breach only the uterine lining, and not the maternal blood vessels, while in most live-bearing squamate reptiles, the embryo adheres to the uterine lining and does not invade (Wu et al., 2011). It is therefore unlikely that preparation for implantation would involve the same basal changes as the rat in all viviparous groups. Instead, a specific set of basal changes may be required for different modes of implantation. As implantation in *S. crassicaudata* is less invasive than that of the rat and no sloughing occurs (Roberts and Breed, 1994), this species is ideal to test the

relationship between uterine changes and implantation mode.

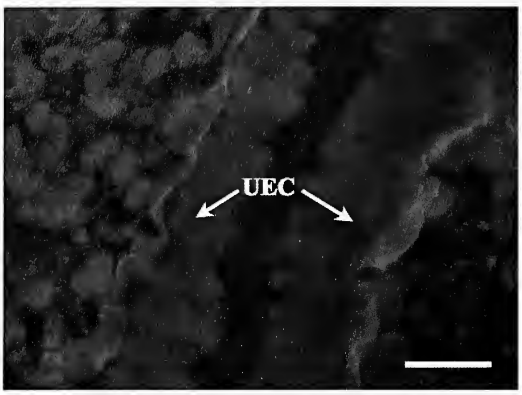

Figure 3: Immunofluorescence micrograph of talin localisation in uterine epithelial cells (UEC) during pregnancy in *Sminthopsis crassicaudata*. A prominent line of talin localisation (green) occurs at the base of both opposing rows of uterine epithelium pre-implantation.
Blue staining=cell nuclei; scale bar = 10μm.

We fluorescently labelled talin and paxillin and identified the patterns of localisation of these molecules in uterine epithelial cells throughout pregnancy in *S. crassicaudata*. We then compared these patterns to those that occur during rat pregnancy. Interestingly, we found that both of these key basal molecules are also involved in pregnancy in *S. crassicaudata*, but the patterns of localisation differ to those of the rat. While talin and paxillin are lost from the uterine lining in the rat before implantation, in preparation for sloughing, these molecules are most tightly localised to the base of the uterine lining during this period in *S. crassicaudata* (Figure 3). This localisation pattern indicates that connections between the uterine lining and underlying tissue are strongest just before the embryo implants.

Different patterns of localisation in uterine cells indicate that these molecules play different roles in marsupial pregnancy compared with rats. As these two species have different modes of implantation, different molecular patterns imply that not all uterine changes in early pregnancy are common, as basal changes differ with implantation mode. Importantly, different molecular patterns in these two species also highlight a fundamental difference in the response of the uterus to the embryo. While loss of the basal molecules talin and paxillin facilitates invasion in the rat (Kaneko et al., 2008, 2009), recruitment of these molecules to the basal plasma membrane of cells lining the uterus appears to strengthen the underlying connections of the uterine lining in *S. crassicaudata*.

Conflict *in utero*

Different uterine responses to the embryo in both the rat and *S. crassicaudata* may be explained in terms of conflict between the mother and the embryo. All vertebrate embryos can manipulate maternal reproductive physiology by releasing hormones and signalling molecules (Crespi and Semeniuk, 2004; Haig, 1993). As the plasma membrane transformation enables intimate contact between embryonic and maternal membranes, embryos can manipulate maternal physiology to a greater extent, and thereby maximise their share of resources (Crespi and Semeniuk, 2004). Manipulation is potentially greatest in species in which the embryo invades the maternal vasculature, including rats and humans, as contact is most intimate (Wooding and Flint, 1994).

Recent evidence suggests that less invasive types of implantation may evolve secondarily from highly invasive implantation in therian mammals through the accumulation of maternal defences to the embryo (Carter, 2008; Crespi and

Semeniuk, 2004). Conflict in utero creates an 'arms race' which results in the evolution of counter strategies on the part of both the mother and embryo to gain control over resource allocation.

The molecular changes in the uterus of *S. crassicaudata*, which has less invasive implantation, support this theory of placental evolution. Reinforcement of the uterine lining, the first barrier to embryonic attachment, just before implantation of the embryo is likely to be an example of a maternal strategy to regulate invasion by the embryo in this species. Hence, this study suggests that conflict occurs *in utero* during marsupial pregnancy, despite the relatively short gestation length in and brief direct contact between the uterus and the embryo.

Identifying additional maternal defences involved in pregnancy in *S. crassicaudata*, and other marsupial species, will allow us to determine the extent to which conflict occurs in the marsupial uterus. Such maternal defences may involve other key molecules which facilitate basal modification of the uterine lining, including members of the integrin molecular family (Kaneko et al., 2011).

In particular, comparison with marsupial species with non-invasive implantation, such as wallabies and kangaroos, will be most informative as maternal defences to the embryo are likely to play a greater role in pregnancy in these species.

Conclusion

While shared morphological changes to the surface of the uterus facilitate attachment of the embryo in both marsupials and placental mammals, this study demonstrates that the molecular responses of the uterus to embryonic invasion differ between mammalian species. The complex response of the marsupial uterus to invasion challenges the assumption that marsupial pregnancy is 'primitive' relative to the placental condition (e.g. Lillegraven, 1975), and highlights the need for these 'alternative' mammals (Renfree, 2010) to be included as a distinct group alongside placental mammals in comparative studies, as both are critical to understanding the evolution of mammalian live birth.

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References

Biazik, J.M., Thompson, M.B., Murphy, C.R. (2007) The tight junctional protein occludin is found in the uterine epithelium of squamate reptiles. J Comp Physiol B, 177(8), 935-943.

Biazik, J. M., Thompson, MB, Murphy, C. R. (2008) Claudin-5 is restricted to tight junction region of uterine epithelial cells in uterus of pregnant/gravid squamate reptiles. Anat Rec, 291, 547-556.

Biazik, J.M., Thompson, M.B., Murphy, C.R. (2010) Desmosomes in the uterine epithelium of non-invasive skink placentae. Anat Rec, 293, 502-512.

Blackburn, D.G., Flemming, A.F. (2009) Morphology, development, and evolution of fetal membranes and placentation in squamate reptiles. J Exp Zool, 312, 579-589.

Brandley, M.C., Young, R.L., Warren, D.L., Thompson, M.B., Wagner, G.P. (2012) Uterine gene expression in the live-bearing lizard, *Chalcides ocellatus*, reveals convergence of squamate and mammalian

pregnancy mechanisms. Genome Biol Evol, 4(3), 394-411.

Carter, A.M., Mess, A. (2007) Evolution of the placenta in eutherian mammals. Placenta, 28, 259-262.

Crespi, B., Semeniuk, C. (2004) Parent-offspring conflict in the evolution of the vertebrate reproductive mode. Amer Nat, 163(5), 635-653.

Enders, A.C., Schlafke, S. (1967) A morphological analysis of the early implantation stages in the rat. Am J Anat, 120, 185-226.

Freyer, C., Renfree, M.B. (2009) The mammalian yolk sac placenta. J Exp Zool, 312(6), 545-554.

Graves, J.A.M. (1996) Mammals that break the rules: Genetics of marsupials and monotremes. Annu Rev Genet, 30, 233-60.

Graves, J.A.M., Westerman, M. (2002) Marsupial genetics and genomics. Trends Genet, 18(10),517-521.

Haig, D. (1993) Genetic conflicts in human pregnancy. Quart Rev Biol, 68, 495-532.

Kaneko, Y., Lindsay, L.A., Murphy, C.R. (2008) Focal adhesions disassemble during early pregnancy in rat uterine epithelial cells. Reprod Fertil Devel, 20, 892-899.

Kaneko, Y., Lecce, L., Murphy, C.R. (2009) Ovarian hormones regulate expression of the focal adhesion proteins, talin and paxillin, in rat uterine luminal but not glandular epithelial cells. Histochem Cell Bio, 132, 613-622.

Kaneko, Y., Lecce, L., Day, M., Murphy, C.R. (2011) β_1 and β_3 integrins disassemble from basal focal adhesions and β_3 is later localised to the apical plasma membrane of rat uterine luminal epithelial cells at the time of implantation. Reprod Fertil Devel, 23, 481-495.

Laird, M.K., Thompson, M.B., Murphy, C.R., McAllan, B.M. (2014) Uterine epithelial cell changes during pregnancy in a marsupial (*Sminthopsis crassicaudata*; Dasyuridae). J Morphol, 275, 1081-1092.

Lillegraven J.A. (1975) Biological considerations of the marsupial-placental dichotomy. Evolution, 29, 707-722.

McAllan, B.M. (2011) "Reproductive endocrinology of prototherians and metatherians", in: Norris, D. O., Lopez, K.H., (eds.) Hormones and Reproduction of Vertebrates, Vol. 5 (Mammals); Elsevier, New York, 195-214.

Murphy, C.R. (2000) Junctional barrier complexes undergo major alterations during the plasma membrane transformation of uterine epithelial cells. Hum Reprod, 15 (Supp. 3), 182-188.

Murphy, C.R. (2004) Uterine receptivity and the plasma membrane transformation. Cell Res, 14(4), 259-267.

Murphy, C.R., Swift, J.G., Mukherjee, T.M., Rogers, A.W. (1982) The structure of tight junctions between uterine luminal epithelial cells at different stages of pregnancy in the rat. Cell Tissue Res, 223, 281-286.

Murphy, C.R., Hosie, M.J., Thompson, M.B. (2000) The plasma membrane transformation facilitates pregnancy in both reptiles and mammals. Comp Biochem Physiol A, 127, 433-439.

Orchard, M., Murphy, C. R. (2002) Alterations in tight junction molecules of uterine epithelial cells during early pregnancy in the rat. Acta Histochem, 104(2), 149-155.

Ramirez-Pinilla, M.P., Parker, S.L., Murphy, C.R., Thompson, M.B. (2012) Uterine and chorioallantoic angiogenesis and changes in the uterine epithelium during gestation in the viviparous lizard, *Niveoscincus conventryi* (Squamata: Scincidae). J Morphol, 273(1), 8-23.

Renfree, M.B. (2010) Marsupials: Placental mammals with a difference. Placenta 31(24), S21-S26.

Roberts, C.T., Breed, W.G. (1994) Embryonic-maternal cell interactions at implantation in the fat-tailed dunnart, a dasyurid marsupial. Anat Rec, 240, 59-76.

Shaw, G., Renfree, M.B. (2006) Parturition and perfect prematurity: birth in marsupials. Aust J Zool, 54, 139-149.

Thompson, M.B., Stewart, J.R., Speake, B.K., Hosie, M.J., Murphy, C.R. (2002) Evolution of viviparity: what can

Australian lizards tell us? Comp Biochem Physiol B, 131, 631-643.

Whittington, C.M., Griffith, O.W., Qi, W., Wilson, A.B. (in review) Male pregnant seahorse transcriptome reveals convergent genes underpinning viviparity.

Wooding, F.B.P., Flint, A.P.F. (1994) "Placentation", in: Lamming, G. E. (ed.) Marshall's Physiology of Reproduction", Part 1. Chapman and Hall, London, 233-460.

Wu, Q., Thompson, M.B.T., Murphy, C.R. (2011). Changing distribution of cadherins during gestation in the uterine epithelium of lizards. J Exp Zool (Mol Dev Evol), 316, 440-45.

Zhang, S., Kong, S., Lu, J., Wang, Q., Chen, Y., Wang, W., Wang, B., Wang, H. (2013). Deciphering the molecular basis of uterine receptivity. Mol Reprod Dev, 80, 8-21.

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Understanding Psychological Responses to Trauma among Refugees: the Importance of Measurement Validity in Cross-cultural Settings

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Abstract

Refugees from the current conflict in Syria have been exposed to a variety of stressors known to increase the risk of mental distress. These may include witnessing atrocities as well as dealing with the challenges of surviving in the displacement context. As a vast array of organisations rush to address mental health outcomes among Syrians, the scientific and conceptual validity of psychological tools used to assess and treat mental health difficulties becomes of paramount importance. Many psychological tools for assessing trauma have been validated in western contexts, but not among Syrians. This paper outlines three errors of reasoning which undermine the validity of psychological methods in cross-cultural contexts, including assuming that western psychiatric categories are universal constructs which can be applied in any context and failing to take contextual factors into account. Qualitative research may help us to better understand culturally specific conceptions of mental health. It is only once we have a solid understanding of how mental distress is understood and expressed among Syrian refugees that we can support effective interventions to alleviate it. The strengthening of indigenous health systems can help promote culturally appropriate mental health care.

Keywords: Syria, Refugee, Psychosocial, Cultural, Assessment, Validity.

Introduction

The current conflict in Syria has led to the deaths of over 200,000 people (IAS, 2014). There are currently approximately 3.7 million registered refugees in surrounding countries (UNHCR, 2015). Many Syrians have been subjected to human rights violations as a result of the conflict (Hassan et al., 2014; Ouyang, 2013). Displaced Syrians face these challenges in the context of living conditions in which it may be difficult to satisfy their basic needs, and where they are isolated from support structures (Taleb et al., 2015).

In this context, a myriad of international actors are seeking to address the psychological needs of Syrians. However, in a rapidly changing environment, how can we be sure that the tools we use to measure and alleviate distress are appropriate? In order to *do no harm*, we must work to validate our tools. While there is pressure to act immediately in a crisis, ensuring the efficacy of action must remain paramount.

The following is a discussion of factors which affect the validity of psychological

measurement tools in humanitarian settings. This discussion is part of an ongoing PhD research program exploring factors affecting uptake and implementation of mental health services among Syrian refugees living in Jordan and Turkey. Our preliminary qualitative research has explored community readiness to address mental health difficulties, cultural factors which influence care-seeking behaviour and culturally specific explanatory models used to understand mental health problems among Syrians living in Jordan. The next phase of our research will build on these foundational concepts with a Train the Trainer approach to build the capacity of a Syrian-founded mental health organisation serving the refugee community in Turkey.

Scientific Validity

Most of the tools used to measure psychological disorders have been developed among western populations (Kleinman, 1988). In fact, most of the categories employed to understand what constitutes normal and abnormal behaviour may represent culture bound constructs which cannot be meaningfully applied in diverse cultural settings (Summerfield, 1999). This calls into question both the conceptual framework and scientific validity of research into psychological health among refugees.

In the field of clinical psychology, establishing the validity of psychological categories and how we measure them can be a complicated process. Firstly, we must define what constitutes psychological disorder. Most experiences associated with psychological disorder exist on a continuum within a population. If we take the example of depression, most people experience sadness at some time in their life. However, some people experience such intense feelings of sadness that they find it difficult to cope. They can no longer go to work or participate in healthy relationships. It is a clinician's job to determine whether a given individual's level of sadness is so severe that it may be the product of a pathological process, understand what this process might be and help the person overcome it. Traditionally, psychologists have sought to define psychological pathology by measuring reported experiences and behaviour within a given population, in order to determine what may be considered *normal*. Experiences which fall at the extreme ends of a given continuum are then defined as *abnormal*. As such, the definition of pathology in the field of psychology is a normative exercise, reflecting the values of the culture in which it operates (De Vos, 2011). The category of *psychological disorder* labels individuals as falling within or without a range which has been classified as *normal* (Plante, 2013).

The purpose of defining and measuring *normality* is so that we can learn more about the underlying processes which contribute to distress. Through the generation of psychological measures, psychologists can discover what kinds of processes are related to psychological disorder. For example, repetitive negative thinking is often associated with depression (Papageorgiou and Wells, 2004), a process for which we now have efficacious, evidence-based treatments (Kenny and Williams, 2007), thereby helping people to overcome depression. The ability of this scientific research to uncover useful constructs relies on the use of valid measures to identify relationships between variables.

Establishing the validity of measures is integral to interpreting empirical data in any discipline. For example, if a biochemist wanted to measure the amount of a certain protein within a sample of tissue, she would require a special tool. She could choose to label the protein with a fluorescent tag which

would light up, enabling her to identify and count the protein. She would first need to ensure that this given tag accurately identifies the protein she is measuring. That is, that her measure is valid. In her field, her data would not be accepted as indicating the presence of the protein unless she used a validated measure. Similarly, in order to be confident that measurement in the field of psychology is accurate, validated measures are required. However, in the case of cross-cultural research, validated measures may not be readily available (Hassan et al., 2014).

Psychological Consequences of War and Displacement

War and displacement can lead to a complex array of negative psychological outcomes (Mollica, 2008) yet mental health among refugees is not clearly understood (Nickerson et al., 2011b; Tol et al., 2011) as psychological research into the effects of trauma is primarily focused on non-refugee western populations (Murray et al., 2010). Estimates of the prevalence of psychological disorder in humanitarian settings have ranged between 0-99% (Steel, 2009). Accurate measurement of prevalence has been hampered by methodological constraints including sample size, sampling procedure (Silove, 1999), and heterogeneous refugee populations (Murray et al., 2010) as well as difficulties in conducting research in crisis situations. Research comparing displaced, war-affected populations to non-refugees indicates elevated levels of psychopathology (Porter and Haslam, 2005), yet there is no psychological treatment for refugees which is firmly supported by a strong evidence base (Crumlish and O'Rourke, 2010; Palic and Elklit, 2011). Research has tended to focus on posttraumatic stress disorder (PTSD). PTSD is a reaction to traumatic experiences characterised by intrusive symptoms, such as re-experiencing the event or nightmares; avoidance of trauma reminders; cognitive and mood alterations, such as memory disturbance, anger, guilt and estrangement; and physiological arousal (APA, 2013).

Trauma leads to a wide variety of sequelae, including effects on brain development (Bellis et al., 2002); cognitive function (Koenen et al., 2003); depression (Cardozo et al., 2004); uncontrollable anger (Brooks et al., 2011); and guilt (Gorman, 2001). In the case of individuals who have experienced ongoing and extreme rights abuses, PTSD may not adequately capture the experience of survivors (Gorst-Unsworth et al., 1993); Herman, 1992). In addition, there is limited research which explores individuals' capacities for resilience during the refugee experience (Hijazi et al., 2014). Conflict-related trauma occurs in a context of disruptions to a variety of social, personal, cultural and political systems which normally promote health. Clinical frameworks for understanding refugee mental health need to take into account impacts on cognitive, interpersonal, social and existential functioning (Nickerson et al., 2011a). A greater focus on a wider range of adaptive functions following trauma may help to ensure that research and treatment accurately address the subjective experience of survivors (Silove, 1999).

Logical Fallacies in the International Application of Western Psychiatric Categories in Diverse Settings

When epidemiologists measure the prevalence of categories like PTSD in humanitarian settings, the interpretation of findings is constrained by the validity of the measures used. In order to arrive at the conclusion that these individuals suffer from the same discrete disease entity as that described in western populations, a number of logical fallacies may have been committed.

Fallacy 1

Arthur Kleinman (1988) identified the category fallacy, the assumption that the identification of symptoms in a different cultural context carries the same significance as it does in western culture. For example, hopelessness in an affluent society in which people have the opportunity to exercise their rights, may be a sign of psychological disorder. However, in a context of continuing loss where "powerlessness is not a cognitive distortion but an accurate mapping of one's place in an oppressive social system" (Kleinman, 1988, pg. 15), hopelessness may be a normal reaction.

Kleinman argued that culturally specific norms inform the way that emotional, cognitive and behavioural phenomena are interpreted, contributing to understandings of what constitutes normal and abnormal within a given society. Each society has its own understanding of the factors which cause distress and psychological pathology. These are explanatory models. These conceptions will, in turn, determine the ways in which distress is expressed. Therefore, each culture will have specific idioms of distress, of which western psychiatric categories are an example.

Since distress may be expressed in a different manner in different cultural contexts, psychological measures which have been validated in one context, may not be valid in another, as items lack cultural relevance and do not include local idioms of distress (Velde et al., 2009). For example, the Beck Depression Inventory (BDI) is a measure of depression which has been validated in numerous western samples (Beck et al., 1988). However, when Nicolas and Whitt (2012) compared qualitative responses of Haitian women to scores on the BDI, they found that these women did not identify with the symptoms on this checklist. That is, the identified symptoms did not carry meaning as expressions of distress within their cultural framework.

Fallacy 2

The assumption that the identification of symptoms associated with PTSD means that individuals have PTSD, may be an example of the fallacy *affirming the consequent.* This error in reasoning takes the form:

If you have PTSD, then you have these symptoms.
You have these symptoms.
Therefore you have PTSD.

Although having PTSD entails having particular symptoms, those symptoms may be the result of causal conditions other than PTSD. For example, recurrent memories and re-experiencing of traumatic incidents may be normative responses in the immediate aftermath of a traumatic event and may in fact be adaptive, as they aid in processing the experience (Gorman, 2001).

Researchers who go into diverse cultural settings and use measurement scales to identify cases of PTSD may be committing this fallacy. The scientifically valid procedure is to first assess the scale for criterion validity in the local context. Criterion validity is established by examining the relationship between scores on the checklist and some external criterion (Van Ommeren, 2003). For example, diagnostic cut-offs for a given checklist are established by comparing scores on the checklist to diagnosis following an in-depth clinical assessment.

Establishing criterion validity in a given community is vital to understanding the contextual factors associated with the identification of a given set of symptoms, and whether or not these symptoms constitute an

abnormal reaction within that society. The blanket use of unvalidated symptom checklists in humanitarian settings may pathologise reactions to stress, for how are we to determine what a *normal* reaction to an extreme situation is (Eisenbruch, 1991)?

Fallacy 3

Another logical problem arises when the identification of symptoms associated with PTSD is taken as evidence to support the conclusion that PTSD is a cross-cultural phenomenon. This may take the form of *begging the question*, a form of logical fallacy in which the truth of the conclusion is assumed in the premise. That is, the person making the argument has assumed that the conclusion they are attempting to prove is self-evident, using it as an axiom to support their argument (Garner, 2001). It is a form of circular reasoning (not to be confused with its incorrect usage to mean "raises the question"). In this case, researchers who employ western-derived measurement instruments to measure PTSD symptoms in diverse cultures and take this as evidence that PSTD is a universal phenomenon, have actually assumed this by applying western categories as if they were self-evident (Summerfield, 1999).

Ethnographic Research can help Validate Assessment Tools

It is circular to apply culture-bound western psychiatric categories (Kirmayer, 2006) as first principles in cross-cultural research. Ethnographic and qualitative research can help us to understand what constitute concepts of "mental" and "health" in local taxonomies. Through this process we can validate the basic assumptions upon which assessment instruments are based (Kleinman, 1988). It is only once we have taken these initial steps that the prevalence of mental disorder in a given context can be established.

A psychiatric ethnography would help to make clear local conceptions of health and disease from the perspective of daily practices and coping strategies. Bolton and Tang (2004) seek to do this by applying ethnographic methods in a rapid assessment participatory model for use in humanitarian settings. They trained local health workers in ethnographic techniques as a primary step to epidemiology and intervention planning. Participants' unconstrained listing of concerns generated a prioritised list of local problems which identified the most pressing psychosocial issues to be discussed in in-depth key informant interviews. The outcomes of this qualitative analysis were used to develop a modification to the Hopkins Symptom Checklist (HSCL) which could measure the prevalence of locally described idioms of distress consistent with depression. In a large randomly selected sample they further found that scores on this checklist were associated with both locally defined measures of functional impairment and western defined criteria for depression (Bolton and Ndogoni, 2000).

The Importance of Identifying Distress

Despite the theoretical limitations raised above, many clinicians seek to apply psychiatric theory in diverse cultures with the aim of achieving practical outcomes (Kirmayer, 2006). The link between traumatic events, such as torture, mental health disorder, such as PTSD or depression, has been demonstrated across a wide range of countries (Steel et al., 2009). Whether or not these categories are always valid, they may often indicate an increased level of distress. Many survivors of trauma do not require

psychological treatment, however it is imperative that treatments are available for people who do (Garcia-Moreno and van Ommeren, 2012). Hopefully, work which seeks to gain a deeper understanding of local healing norms (for example the work of Al-Krenawi and Graham (2000), Hinton et al. (2009), Mollica et al. (1993)) can assist in identifying individuals in need of assistance.

Some argue that, while valid, these theoretical issues have led to polarisations which risk obscuring practical realities for the severely mentally ill (Kirmayer, 2006; Silove et al., 2000). However mental disorder is classified, the fact remains that across cultures, a subset of people suffer marked functional and social impairment as a result of mental health difficulties (Kleinman, 1988), most notably among those with severe problems such as psychosis, neurological disorder and epilepsy (Silove et al., 2000). The mentally ill are at increased risk in crisis situations. For example, when a psychiatric hospital in Aleppo, Syria, was bombed in 2012, patients had to flee and were left without support. There is evidence that some of these patients were subsequently killed by sniper fire while wandering the streets (Abou-Saleh and Mobayed, 2013).

Identifying Distress Among Syrians

In order to appropriately diagnose and treat mental health issues among Syrian refugees, it is necessary to understand how they perceive and describe mental health problems (Tol et al., 2011). There are, however, no standard clinical instruments for assessing trauma which have been validated in Syrian populations (Hassan et al., 2014). In fact, psychiatric services have historically not been widely available in Syria. For example, in 2012 there were <0.5 psychiatrists, 0 psychologists and 0.5 psychiatric nurses per 100,000 population in Syria (Okasha et al., 2012). Prior to 2011, available services were generally residential and restricted to major cities (1,200 beds) (Abou-Saleh and Mobayed, 2013). In addition, public health systems have come under attack in Syria and are no longer fully functional (Kherallah et al., 2015).

In addition to having limited practical access to treatment options, stigma may prevent individuals from seeking help. There is limited research on the impact of stigma among Syrians in particular, however, a review of 22 publications of psychological interventions adapted for Arabic speaking patients reported that a high number of papers identified fear of stigma as a barrier to care (Gearing et al., 2013). Arabic speaking people interviewed in Sydney reported that having a heritable disease (such as schizophrenia) may be considered appropriate grounds for divorce and 51% said that isolating people with mental health disorders was considered normal (Youssef and Deane, 2006). Fear of social consequences may lead to disclosure of somatic symptoms only (Weiss et al., 2001) and patients may be unlikely to attend dedicated mental health clinics for fear that they will be observed. Provision of mental health services in the primary health care context may help to overcome this (Nasir and Al-Qutob, 2005).

While it is important to ensure that professional help is available to those who would like it, Syrians may have alternative ways of coping with distress with which they identify more strongly. For example, Syrian refugees in southern Turkey reported reasons for not seeking care, including only needing God, preferring to speak to family or friends and stating that their emotional reaction to the circumstance is normal, so they do not require specialised treatment (Jefee-Bahloul et al., 2014). It is possible that members of the

Syrian refugee community are best placed to understand the mental health needs of their compatriots. In which case, interventions which work to strengthen indigenous coping systems may be an effective means to overcome validity concerns in translating cultural conceptions of distress.

Rebuilding Community Structures

Silove and colleagues (Silove, 1999) identify how the breakdown of systems of social networks, justice and other support structures in post-conflict settings undermines community structures which might otherwise provide support to individuals. Programs which help to rebuild these structures can promote healing at the community and individual level. For example, greater trust in the community and a sense of community cohesion have been associated with better social support and reductions in mental health difficulties in a longitudinal study of displaced children in Burundi, indicating that programs which build a sense of community may help children to marshal social resources in order to improve health (Hall et al., 2014).

Situations of dependency associated with living in refugee camps, or lack of recognition of previous roles and qualifications in resettlement countries can lead to major disruptions to individuals' identity (Silove, 1999). Involvement in meaningful action leading to recognition as a valuable member of the community has been identified by refugees as contributing to recovery from PTSD following conflict and displacement (Ajdukovic et al., 2013).

Conclusions

For humanitarian organisations, mental health practitioners and scientific researchers working in conflict and post-conflict settings, questions of validity cannot be overlooked when applying empirically based methods to provide care to individuals who have experienced considerable adversity. However, there are limited opportunities to establish validity in the context of humanitarian crises. One solution to this problem may be to employ measurement methods which have been validated in different contexts, and hope that they produce meaningful data. An arguably superior solution is to take advantage of the skill and understanding of people within the local community. The detailed cultural knowledge of these individuals enables them to make valid assessments of distress, whether conducting clinical assessments or research. While some members of refugee communities will be in need of assistance in coping with the experiences of war and displacement, others are likely to be resilient. These individuals may be in a position to play a leadership role in rebuilding community support systems. All humanitarian organisations are in a position to support these leaders to facilitate the generation of culturally appropriate psychosocial programs. Respecting their knowledge engenders respect and human dignity.

References

Abou-Saleh, M. & Mobayed, M. (2013) Mental Health in Syria, *International Psychiatry*, 10, 3, 58-60.

Ajdukovic, D., Ajdukovic, D., Bogic, M., Franciskovic, T., Galeazzi, G. M., Kucukalic, A., Lecic-Tosevski, D., Schützwohl, M. & Priebe, S. (2013) Recovery from Posttraumatic Stress Symptoms: A Qualitative Study of Attributions in Survivors of War, *PLoS ONE*, 8, 8.

Al-Krenawi, A. & Graham, J. R. (2000) Culturally Sensitive Social Work Practice With Arab Clients in Mental Health Settings, *Health & Social Work*, 25, 1, 9-22.

APA (2013) *The Diagnostic and Statistical Manual of Mental Disorders: DSM 5*, bookpointUS.

Beck, A. T., Steer, R. A. & Carbin, M. G. (1988) Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation, *Clinical Psychology Review*, 8, 1, 77-100.

Bellis, M. D. D., Keshavan, M. S., Shifflett, H., Iyengar, S., Beers, S. R., Hall, J. & Moritz, G. (2002) Brain Structures in Pediatric Maltreatment-Related Posttraumatic Stress Disorder : A Sociodemographically Matched Study, *Biological Psychiatry,* 52(11), 1066-1078 .

Bolton, P. & Ndogoni, L., (2000) Cross-Cultural Assessment Of Trauma-Related Mental Illness, *CERTI,* 71-71.

Bolton, P. & Tang, A. M. (2004) Using ethnographic methods in the selection of post-disaster, mental health interventions, *Prehospital and disaster medicine : the official journal of the National Association of EMS Physicians and the World Association for Emergency and Disaster Medicine in association with the Acute Care Foundation*, 19, March, 97-101.

Brooks, R., Silove, D., Steel, Z., Steel, C. B. & Rees, S. (2011) Explosive anger in postconflict Timor Leste: interaction of socio-economic disadvantage and past human rights-related trauma, *Journal of affective disorders*, 131, 1-3, 268-76.

Cardozo, B. L., Bilukha, O. O., Crawford, C. a. G., Shaikh, I., Wolfe, M. I., Gerber, M. L. & Anderson, M. (2004) Mental health, social functioning, and disability in postwar Afghanistan, *JAMA*, 292, 5, 575-84.

Crumlish, N. & O'Rourke, K. (2010) A systematic review of treatments for post-traumatic stress disorder among refugees and asylum-seekers, *The Journal of Nervous and Mental Disease*, 198, 4, 237-251.

De Vos, J. (2011) The psychologization of humanitarian aid: skimming the battlefield and the disaster zone, *History of the Human Sciences*, 24, 103-122.

Eisenbruch, M. (1991) From post-traumatic stress disorder to cultural bereavement: Diagnosis of Southeast Asian refugees, *Social Science & Medicine*, 33, 6, 673-680.

Garcia-Moreno, C. & van Ommeren, M. (2012) Mental health and psychosocial support for conflict-related sexual violence : 10 myths, *World Health Organisation,* 2.

Garner, B. A. (2001) *A Dictionary of Modern Legal Usage*, Oxford University Press.

Gearing, R. E., Schwalbe, C. S., MacKenzie, M. J., Brewer, K. B., Ibrahim, R. W., Olimat, H. S., Al-Makhamreh, S. S., Mian, I. & Al-Krenawi, A. (2013) Adaptation and translation of mental health interventions in Middle Eastern Arab countries: a systematic review of barriers to and strategies for effective treatment implementation, *The International journal of social psychiatry*, 59, 7, 671-81.

Gorman, W. (2001) Refugee Survivors of Torture: Trauma and Treatment, *Professional Psychology: Research and Practice*, 32, 5, 443-451.

Gorst-Unsworth, C., Van Velsen, C. & Turner, S. (1993) Prospective Pilot Study of Survivors of Torture and Organized Violence: Examining the Existential Dilemma, *The Journal of Nervous and Mental Disease*, 181, 4, 263-264.

Hall, B., Tol, W., Jordans, M., Bass, J. & de Jong, J. (2014) Understanding resilience in armed conflict: Social resources and mental health of children in Burundi, *Social Science and Medicine*, 114, 121-128.

Hassan, G., Quosh, C., Mekki-berrada, A., Youssef, A., Coutts, A. & Kirmayer, L. (2014) *Culture and Mental Health of Syrians A primer for mental health professionals working with Syrian refugees and displaced people, UNHCR.*

Herman, J. L. (1997) *Trauma and Recovery*, BasicBooks.

Herman, J. L. (1992) Complex PTSD: A syndrome in survivors of prolonged and repeated trauma, *Journal of Traumatic Stress*, 5, 3, 377-391.

Hijazi, A. M., Lumley, M. A., Ziadni, M. S., Haddad, L., Rapport, L. J. & Arnetz, B. B. (2014) Brief Narrative Exposure Therapy for Posttraumatic Stress in Iraqi Refugees : A Preliminary Randomized Clinical Trial, *Journal of Traumatic Stress*, 27(3), 314-322.

Hinton, D. E., Hofmann, S. G., Pollack, M. H. & Otto, M. W. (2009) Mechanisms of Efficacy of CBT for Cambodian Refugees with PTSD: Improvement in Emotion Regulation and Orthostatic Blood Pressure Response, *CNS Neuroscience & Therapeutics*, 15, 3, 255-263.

IAS (2014), *I am Syria,* www.iamsyria.org/daily-death-count.html.

Jefee-Bahloul, H., Moustafa, M. K., Shebl, F. M. & Barkil-Oteo, A. (2014) Pilot assessment and survey of syrian refugees' psychological stress and openness to referral for telepsychiatry (PASSPORT Study), *Telemedicine journal and e-health : the official journal of the American Telemedicine Association*, 20, 10, 977-9.

Kenny, M. a. & Williams, J. M. G. (2007) Treatment-resistant depressed patients show a good response to Mindfulness-based Cognitive Therapy, *Behaviour research and therapy*, 45, 617-625.

Kherallah, M., Alahfez, T., Sahloul, Z., Eddin, K. D. & Jamil, G. (2015) Health care in Syria before and during the crisis, *Avicenna Journal of Medicine*, 2(3), 51.

Kirmayer, L. J. (2006) Beyond the 'new cross-cultural psychiatry': cultural biology, discursive psychology and the ironies of globalization, *Transcultural Psychiatry*, 43, March, 126-144.

Kleinman, A. (1988). *Rethinking psychiatry: From cultural category to personal experience.* Simon and Schuster.

Koenen, K. C., Moffitt, T. E., Caspi, A., Taylor, A. & Purcell, S. (2003) Domestic violence is associated with environmental suppression of IQ in young children, 15, 297-311, *Development and psychopathology*, 15(02), 297-311.

Mollica, R. F. (2008) *Healing Invisible Wounds: Paths to Hope and Recovery in a Violent World*, Vanderbilt University Press.

Mollica, R. F., Donelan, K., Tor, S., Lavelle, J., Elias, C., Frankel, M. & Blendon, R. J. (1993) The effect of trauma and confinement on functional health and mental health status of Cambodians living in Thailand-Cambodia border camps, *JAMA : the Journal of the American Medical Association*, 270, 581-586.

Murray, K. E., Davidson, G. R. & Schweitzer, R. D. (2010) Review of refugee mental health interventions following resettlement: best practices and recommendations, *American Journal of Orthopsychiatry*, 80, 4, 576-85.

Nasir, L. S. & Al-Qutob, R. (2005) Barriers to the diagnosis and treatment of depression in Jordan. A nationwide qualitative study, *The Journal of the American Board of Family Practice*, 18, 2, 125-131.

Nickerson, A., Bryant, R. A., Brooks, R., Steel, Z., Silove, D. & Chen, J. (2011a) The familial influence of loss and trauma on refugee mental health: a multilevel path analysis, *Journal of Traumatic Stress*, 24, 1, 25-33.

Nickerson, A., Bryant, R. A., Silove, D. & Steel, Z. (2011b) A critical review of psychological treatments of posttraumatic stress disorder in refugees, *Clinical Psychology Review*, 31, 3, 399-417.

Nicolas, G. & Whitt, C. L. (2012) "Conducting qualitative research with a Black immigrant sample: Understanding depression among Haitian immigrant women.", in Nagata, D., Kohn-wood, L. & Suzuki, L. A. (eds.) *Qualitative Strategies for Ethnocultural Research*, American Psychological Association, Washington, 199-217.

Okasha, A., Karam, E. & Okasha, T. (2012) Mental health services in the Arab world, *World Psychiatry*, 11, 1, 52-54.

Ouyang, H. (2013) Syrian refugees and sexual violence, *The Lancet*, 381, 9884, 2165-2166.

Palic, S. & Elklit, A. (2011) Psychosocial treatment of posttraumatic stress disorder in adult refugees: A systematic review of prospective treatment outcome studies and a critique, *Journal of Affective Disorders*, 131, 1-3, 8-23.

Papageorgiou, C. & Wells, A. (2004) Depressive rumination, *Nature, Theory and Treatment.*

Plante, T. (2013) *Abnormal Psychology Across the Ages*, ABC-CLIO.

Porter, M. & Haslam, N. (2005) Predisplacement and postdisplacement factors associated with mental health of refugees and internally displaced persons: a meta-analysis, *JAMA*, 294, 5, 602-12.

Silove, D. (1999) The Psychosocial Effects of Torture, Mass Human Rights Violations, and Refugee Trauma: Toward an Integrated Conceptual Framework, *The Journal of Nervous and Mental Disease*, 187, 4, 200-207.

Silove, D., Ekblad, S. & Mollica, R. (2000) The rights of the severely mentally ill in post-conflict societies, *Lancet*, 355, 1548-1549.

Steel, Z., Chey, T., Marnane, C., Bryant, R. A. & Ommeren, M. V. (2009) Association of Torture and Other Potentially Traumatic Events with Mental Health Outcomes Among Populations Exposed to Mass Conflict and Displacement. A Systematic Review and Meta-analysis, *Journal of the American Medical Association*, 302, 5, 537-549.

Steel Z, C. T. S. D. M. C. B. R. A. v. O. M. (2009) Association of torture and other potentially traumatic events with mental health outcomes

among populations exposed to mass conflict and displacement: A systematic review and meta-analysis, *JAMA: The Journal of the American Medical Association*, 302, 5, 537-549.

Summerfield, D. (1999) A critique of seven assumptions behind psychological trauma programmes in war-affected areas, *Social Science & Medicine*, 48(10), 1449-1462.

Taleb, Z. B., Bahelah, R., Fouad, F. M., Coutts, A., Wilcox, M. & Maziak, W. (2015) Syria: health in a country undergoing tragic transition, *International journal of public health*, 60 Suppl 1, 63-72.

Tol, W. A., Patel, V., Tomlinson, M., Baingana, F., Galappatti, A., Panter-Brick, C., Silove, D., Sondorp, E., Wessells, M. & van Ommeren, M. (2011) Research Priorities for Mental Health and Psychosocial Support in Humanitarian Settings, *PLoS Med*, 8, 9, e1001096.

UNHCR (2015) *Syria Regional Response Plan*, UNHCR.

Van Ommeren, M. (2003) Validity issues in transcultural epidemiology, *British Journal of Psychiatry*, 182, 376-378.

Velde, J. V. D., Williamson, D. L. & Ogilvie, L. D. (2009) Participatory Action Research: Practical Strategies for Actively Engaging and Maintaining Participation in Immigrant and Refugee Communities, *Qualitative Health Research,* 19(9), 1293-1302..

Weiss, M. G., Jadhav, S., Raguram, R., Vounatsou, P. & Littlewood, R. (2001) Psychiatric stigma across cultures: Local validation in Bangalore and London, *Anthropology & Medicine,* 8, 1, 71-87.

Youssef, J. & Deane, F. P. (2006) Factors influencing mental-health help-seeking in Arabic-speaking communities in Sydney, Australia, *Mental Health, Religion & Culture*, 9, 1, 43-66.

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Single-cell Isolation Devices: Understanding the Behaviour of Cells

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Abstract

Practical usefulness in biological and clinical settings has become an important focus during the development and implementation of new instrumentation and assays. These developments have allowed it to become possible to determine gene- and protein-content, as well as mutations within the transcriptome of a single cell. In order to be able to reach the full potential of the available instrumentation and assays, it is required to develop a method to first isolate an individual cell. This review serves as an overview of available techniques for single-cell isolation by describing the biological information about a single cell that can be obtained from each technique.

Introduction

The invention of the microscope in 1676 by Anton van Leeuwenhoek introduced the concept of studying how the human body is constructed. From here, Robert Hooke coined the term "cell" as the basic building block for all living species. The discovery of the cell was instrumental to further our knowledge about many aspects of the human body as focus was then shifted from whole tissue to cell suspensions so that analysis could be undertaken on cells with prior knowledge of their origin. The development of the first immortal cell line in 1951, the HeLa cell line, showed that it was possible to investigate cells with respect to time to better understand how the cells respond to a particular treatment. Using this understanding of how to encourage the continuous culture of cells, a wide range of cell cultures originating from various parts of the human body were created to learn how different parts of the human body react to external influences (Norris and Ribbons, 2006). From these cell lines, it has been noticed that mammalian cells from the same cell line can respond differently to the same procedures to analyse them (Andersson Svahn and van den Berg, 2007). This means that information collected from cell populations represents averaged values and can potentially mask rare but important events (Di Carlo and Lee, 2006). This has led to a shift from studying cells within cell lines down to individual cells in order to learn how each cell behaves and communicates with its neighbours. A cell can for example be monitored as it migrates or divides into two cells. The understanding of cell migration would give insights into the nature of tissue repair after injury whilst cell division is of interest in for example, cancer, due to the uncontrolled

rate of cell proliferation of a cancer cell compared to a normal cell. Furthermore, the dynamic study of living cells can increase the understanding of the interconnecting molecular events continually taking place in each cell as it responds to external influences such as a particular treatment or other cells. As the human body contains a variety of cells such as stem cells, blood cells and tissue cells, that all vary in their behaviour, a wide range of single cell devices has been developed that enables behavioural information for all types of cells to be gained.

Methods for Single-Cell Isolation

Serial Dilution

As with any problem facing concentrations or amounts that are too high, the first solution that tends to come to mind is to dilute the sample. This was no different with cells, with the first methods to reach lower numbers of them being achieved by successively diluting cell solutions until it was possible to microscopically observe the occasional aliquot that contained one cell. However, tracking these single cells in bulk amounts of volumes required to perform serial dilution can be difficult and therefore analysing these cells may not be possible. For this reason, a method to trap these cells is required so that continuous analysis of them is possible.

Microwell Trapping

Once a cell-rich sample had been diluted such that an aliquot containing a single cell was made available, a means to be able to investigate the behaviour of that cell is required. In other words, a method to trap the individual cells is required. The most common method to trap cells is by placing them in an array of wells. Since most of these cell lines rely on the cells adhering to a surface in order to proliferate, they can be easily identified once they have adhered to the bottom of the well. The physical wall placed around each sample to protect it from cross-contamination with other samples allows for multiple parallel studies to be undertaken within each adjacent well, increasing the amount of information that can be obtained. As the number of cells to be studied decreases, so do the associated volumes. To facilitate the smaller volumes used, wells of continuously decreasing size are being developed. At present, the most commonly used is a 96-well plate. With these wells having a surface area of 0.32 cm^2 (about 100 000 times the area of an adhered cell), they are more suited to the study of small colonies (hundreds of cells) rather than an individual cell.

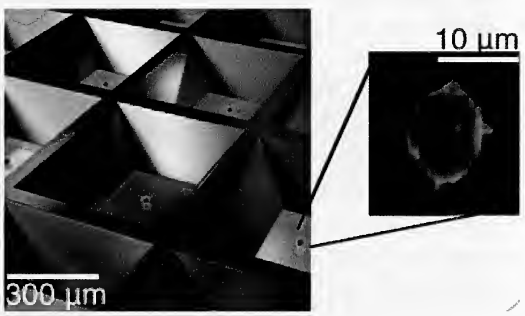

Figure 1: A representation of microwells containing single cells and (inset) a microscope image of an adhered human stem cell. Reproduced from Lindstrom et al. (2009).

The logical progression is to make wells that are similar in size to that of the spread cell (Figure 1). Despite its miniaturisation, this approach is still quite simple and is therefore quite popular with overviews of the varying methods being reviewed in (Walling and Shepard 2011, Lindstrom and Andersson-Svahn 2011). The smaller sizes and volumes within these microwells mean

that shorter diffusion distances are present and therefore the immediate effect of an external influence on a particular cell can be examined. Furthermore, these wells have been created with varying characteristics such as well shape, rounded (Wood et al. 2010, Rettig and Folch 2005, Tokimitsu et al. 2007, Ostuni et al. 2001), hexagonal (Taylor and Walt 2000, Deutsch et al. 2006) and square (Chin et al. 2004, Revzin et al. 2005, Lindstrom et al. 2009), number of wells (100's: Taylor and Walt 2000, Ostuni et al. 2001, Lindstrom et al. 2009; 10,000's: Chin et al. 2004, Revzin et al. 2005, Rettig and Folch 2005, Deutsch et al. 2006; or 100, 000's: Tokimitsu et al. 2007) and fabrication material (glass: Deutsch et al. 2006, Lindstrom et al. 2009; silicon: Tokimitsu et al. 2007; polydimethylsiloxane (PDMS): Rettig and Folch 2005, Ostuni et al. 2001; and polyethylene glycol (PEG): Revzin et al. 2005).

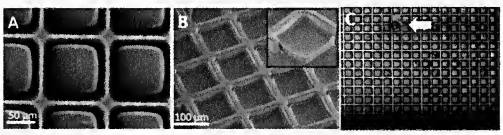

Figure 2: (A) Brightfield and (B) SEM images of microrafts. Inset shows a side view of a raft with PDMS partially removed. (C) Removal of a predetermined microraft after cell seeding. Reproduced from Gach et al. (2011) with permission from AIP Publishing LLC.

Part of the driver for these different variables is finding conditions for the optimisation of cell viability over moderately longer time periods, enabling the viewer to gain information with regards to the effects of a particular external influence over a period of 8 days. Despite it being desirable for aiding the initial colonisation of a single cell, the small surface area of the microwells does not facilitate the growth of these colonies and cell viability decreases as overcrowding in each well occurs. Since many influences can cause the cells to display symptoms only months, or even years after they are affected, the ability to analyse these cells over much longer time periods is required.

Cell Microsystems (North Carolina) had this in mind when they created their IsoRaft system, which is a microwell plate made of a compliant polymer substrate. In the bottom of each well is a concave-shaped tile-like object made of hard polymer-material (polystyrene or epoxy resin) that has been called a microraft (Figure 2A and B). This concave-like shape of the microraft causes the cells to adhere at the bottom when they are trapped in the microwells. Much like the other microwell methods, a single cell can be selected and analysed to determine the immediate effects of an external influence on the behaviour of the cell. For long-term effects, a selected cell can be monitored until it forms a small colony. Once the microraft begins to get crowded with cells, a needle can then be inserted into the compliant polymer substrate adjacent to the microraft containing that colony, moved around it and in doing so, removing the microraft containing the colony of interest from the microwell (Figure 2C) (Wang et al. 2010). However, an issue faced when using this IsoRaft system was the difficulty associated with trying to recover this released microraft. Cell Microsystems overcame this issue by incorporating magnetic nanoparticles[1] into the microrafts and then collecting them magnetically (Gach et al. 2011). These collected microrafts

[1] Magnetic nanoparticles are particles with a diameter of less than 100 nm and contain a magnetic iron oxide core. This magnetic core allows them to be recovered using a magnet.

(containing the cells of interest) can then be placed into a flask with a larger surface area than that of the microwells. This larger surface area allows for more replication cycles and therefore more long-term (up to years) effects, such as a better understanding of the mutation rates of cells. However, these methods typically focus on the behavioural analysis of these cells. This is because limited techniques, with the exception of cytoplasmic staining, can be used to gain structural information about cells that are trapped on a surface.

Microwells have shown to be a simple and effective method to isolate an individual cell. The next step is to be able to provide a technique that can acquire the individual cells in an unbound state such that the newly-developed assays and instruments can be used to gain further information about the behaviour and contents of single cells as they are exposed to an external influence.

Droplet Trapping

One demonstrated way to track single cells in an unbound state is by enclosing them in droplets of low volumes (fL to nL), forming micro-chambers for individual reactions. High-frequency (Hz-kHz) droplet generators in microfluidic devices [2] form monodisperse drops of water in an inert and immiscible carrier fluid (oil). Controlling the number of loaded cells per drop has been a barrier for droplet-based single-cell analysis, due to the stochastic limitations of single-cell loading resulting in *ca.* 30% of single-cell occupancy (similar to many microwell approaches). A demonstrated way to overcome this limitation has been to evenly space cells in a microchannel to make sure that the cells entered the drop generator with the same frequency as drop formation (Figure 3; Edd et al. 2008). As with limited dilution in general arraying techniques, empty droplets are often preferred rather than overloading droplets with several cells. The droplets can thereafter be merged with other droplets, (Chabert et al. 2005) split into two (Link et al. 2004) or dielectrophoetic (DEP) sorted (Ahn et al. 2006).

Figure 3: A micrograph depicting the encapsulation of single cells within nanodroplets. The ordering of cells entering the droplet chamber increases the likelihood of a droplet containing a single cell. Scale bar = 150μm. Reproduced from Joensson and Svahn (2012) with permission from Wiley and Sons.

There are two strong advantages of this technique: 1) since each cell is kept within its own separate droplet, isolated from other droplets, the risk of cross-contamination decreases and 2) the even lower volume of liquid surrounding each encapsulated cell when compared to microwells, results in even more accurate short-term information. Weitz and co-workers showed an example of such an application by incubating single hybridoma in 33 pL drops of media, giving rise to secreted detectable concentrations of antibodies after 6h (Koester et al. 2008). Another example of an application of cell encapsulation demonstrates laser-induced cell lysis within droplets followed by monitoring the activity of β-galactosidase enzyme from a single cell (He et al. 2005). Samuels and co-workers (Brouzes et al. 2009) have extended on these applications

[2] A microfluidic device involves fabricating chambers with micrometre dimensions that are designed to accurately control the flow of volumes of liquids in the millilitre range.

with an integrated droplet-based workflow for conducting a mammalian cell cytotoxicity screen at high throughput. Cells were kept viable for four days (though cell proliferation was only detected during the first 24h) and a drug library was screened for their cytotoxic effects against cells from a myeloid cell line. Most importantly, the unbound nature of these single cells within a droplet allows structural information to be gained from them. Enzyme amplification was used to detect low abundance cell-surface biomarkers, CD19 and CCR5 on single U937[3] cells (Joensson et al. 2009) and shows the potential of this technique to be used to decipher the expression of genes, and even potentially, mutations within these genes as the cells become affected. In order to get more accurate information about cells with respect to time, issues such as 1) changes in the droplets such as coalescence, nutrient depletion or the accumulation of toxic metabolites are obstacles that need to be considered before robust analyses over longer periods of time can be achieved. Despite minor success with regards to this (Clausell-Tormos et al. 2008) these issues still hinder most methods that have adopted this technique and 2) the fact that each cell is isolated as many influences affect the way that a cell responds to its environment and communicates with its neighbouring cells. Merging droplets can gain information with regards to this but with limited control over which droplets to merge, this information can only be elementary. In essence, droplet trapping has served as a powerful means for being able to gain short term information about the behaviour or contents of a single cell. The next step is to be able to get information that more accurately mimics the conditions within the human body. Namely, the behaviour of a single cell within a network of cells over extended periods of time.

[3] U937 cells are a commonly used cell line used in biomedical research. They were isolated from the histiocytic lymphoma of a 37-year-old male patient.

Hydrodynamic Trapping

These requirements are addressed by hydrodynamic trapping. This technique involves flowing a cell solution through a microchannel that contains microstructures that trap individual or clusters of cells. The flow of medium through the channel after the cells have been trapped means that the nutrients for the cells are being replenished, allowing for the cells to be kept in a viable state for longer periods of time. Furthermore, hydrodynamic trapping has shown a high selectivity when it comes to pairing individual cells together to see how cells interact with each other when placed in a range of environments. It comes on the back of initial work carried out by Lee and co-workers (Di Carlo et al. 2006b), who first showed that it was possible to generate single-cell arrays using U-shaped hydrodynamic trapping structures with geometries that are biased to trap only single cells (Figure 4A). Lee and co-workers (Di Carlo et al. 2006a) used these U-shaped arrays to report novel data on the single-cell concentration distribution of carboxylesterases within three different human cell lines, as well as on the inhibition of intracellular esterases by the non-specific inhibitor nordihydroguaiaretic acid. Benavente-Babace and co-workers (Benavente-Babace et al. 2014) further showed that it was possible to treat a subpopulation of the single cells captured with these U-shaped geometries. From these initial studies, hydrodynamic traps have been used to capture pairs of single cells (Figure 4B) to gain a further

understanding of cell-cell interactions such as cell fusion (Skelley et al. 2009) and cellular uptake of secreted proteins from neighbouring cells (Chen et al. 2014). This information can be coupled to single-cell studies and can give valuable insights into how a disease requires the presence of regular cells in order to be active.

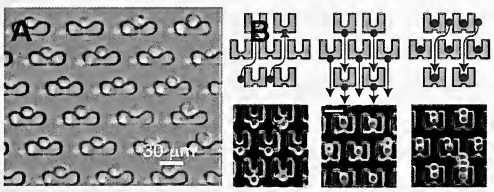

Figure 4: (A) Arrayed single-cell culture within U-shaped sieves. Reproduced from Di Carlo et al. (2006a) with permission from the American Chemical Society.
(B) Paired single 3T3[4] cells within a U-shaped sieve. Green-stained cells are first loaded 'up' towards the smaller back side capture cup (left panel). The direction of the flow is then reversed and the cells are transferred 'down' into the larger front-side capture cup two rows below (middle panel). The red-stained cells are then loaded in from the top and cells are captured above the first cell type (right panel). Scale bar is 30μm. Reproduced from Skelley et al. (2009) with permission from Nature Publishing Group.

The hydrodynamically-based trapping methods discussed thus far show that it is possible to study the interactions between two individual cells of a different type. Zhang et al. (2014) have improved on the modularity of this method by creating a hand-held single-cell pipette. The pipette contains positive pressure and negative pressure channels, along with a tip that contains associated channels. Single-cell transfer is achieved through four steps: preparation, capture, washing and release (Figure 5D). Initially, the channels and tip are filled with cell-free medium. Cells are then sucked up into the tip where a single cell is captured by a hook located within it (Figure 5A) whilst the pipette is quickly transferred to cell-free medium to wash the remaining cells into the negative pressure channel. The captured single cells are easily released into nanolitre droplets by applying a gentle pushing force to the positive pressure channel. Subsequently, single-cell droplets are conveniently transferred into designated containers, such as standard 96-/384-well plates, Petri dishes, and vials.

The versatility of this hand-held single-cell pipette means that any single cell can be isolated and then its genetic and cytoplasmic contents can be determined or multiple single cells can be placed next to each other to gain knowledge about how a network of cells communicates.

These single-cell methods showed that it was possible to trap single or paired cells. To build from this, the ability to get behavioural and structural information from these isolated individual cells lies within the development of newly-developed instruments and assays.

[4] 3T3 cells are a fibroblast cell line derived from Swiss albino mouse embryo tissue.

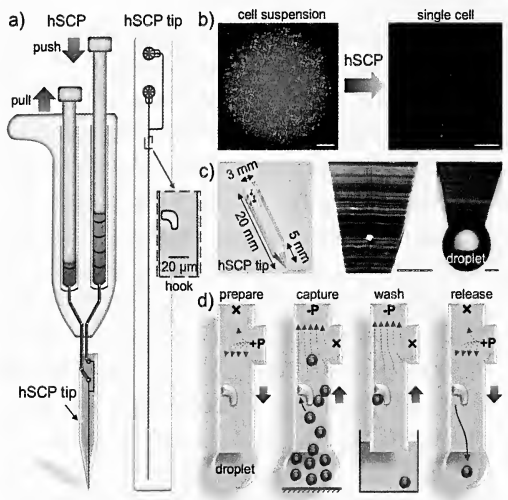

Figure 5: Design and mechanism of the hand-held single-cell pipette (hSCP).
(a) The hSCP involves one dual-channel pipette and one hSCP tip. A magnified hook for single-cell capture is shown.
(b) A single calcein-labeled SK-BR-3 cell is isolated directly from a dense cell suspension by hSCP.
(c) The hSCP tip with a conical end and two magnified tip ends shown before and after extrusion of aqueous solution.
(d) Work flow for single-cell isolation using hSCP. Scale bar in (b) and (c) = 20μm. Reproduced from Zhang et al. (2014) with permission from the American Chemical Society.

Single-cell Analysis Instruments and Assays

Due to the important nature of the information that can be gained, several single cell analysis methods have become available. Most of the available protocols are focused on either the nucleic acid content (PCR-based methods) or on cytoplasmic protein level (cytometric-based methods).

Polymerase Chain Reaction (PCR)-based Analysis

PCR is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. This amplification process has allowed it to become a powerful technique for the genetic screening for the small numbers of cells, towards the single cells isolated from single-cell isolation devices.

To be able to get a further understanding of the structural make-up of individual cells as they are exposed to an external influence, it is required to not only increase the number of copies of a particular gene, but also to increase the number of genes to be amplified. For this reason, gene analysis has leaped forward in the mid 1990s with the development of new amplification methods such as single-primer isothermal amplification (SPIA) (Ma et al. 2013) and the rise of high-throughput RNA/DNA sequencing, or RNA/DNA-seq, which gives the sequences of thousands of cellular RNAs/DNAs from a single cell at once, giving rise to a new field of single cell sequencing. Due to its high impact, the field has seen a proliferation of methods for performing single-cell RNA/DNA-seq (Hashimshony et al. 2012, Tang et al. 2009, Ramskold et al. 2012, Islam et al. 2011, Sasagawa et al. 2013) and have been reviewed in detail (Sandberg 2014, Tang et al. 2011). More detailed information about the behaviour of cancerous cells has already been obtained, such as the re-evaluation of their mutation rates. Bulk-sequencing studies have estimated that the mutation rate across many human cancers is, on average 210-fold higher than normal cells (Bielas et al. 2006, Bielas and Loeb 2005). However, single cell sequencing has shown

that an endoplasmic reticulum-positive breast cancer cell did not have an increased mutation rate relative to that of normal cells, whereas a triple negative (endoplasmic reticulum, pathogenesis-related and human epidermal growth factor receptor 2) breast cancer cell showed an approximately 10X increase (eight mutations per cell division) relative to that of normal cells (Wang et al. 2014). Furthermore, differences within the transcriptional profiles of breast cancer samples taken from patients have been recorded (Powell et al. 2012), point mutations within targeted genes identified (Heitzer et al. 2013) and whole genome sequencing of single cancerous cells to trace how the tumour evolves (Navin et al. 2011).

Despite the important information that PCR-based methods have already supplied, the amount concentration of a gene is only in the order of 10^{-12} M (~10 pg per cell) while the total protein content is as high as 10^9 molecules per cell (hundreds of pg). It has been estimated that a cell contains more than 100,000 different proteins, ranging from <200 copies of many receptors, 1,000–10,000 copies of signalling enzymes, to 10^8 copies of some structural proteins (Cooper and Hausman 2007). For this reason, limiting the investigation to just the genes that are present in the nucleus results in only a partial understanding of the structural make-up of individual cells.

Cytometric-based Analysis

Cytometry involves measuring the characteristics of cells, focusing on the enumeration and understanding of specific proteins on the cell surface or in the cytoplasm. For this reason, cytometric-based analytical methods are considered a suitable avenue to broaden an investigation of single cells beyond their nucleic material.

Image cytometers involve the use of microscopes to acquire highly-resolved images of the single cells isolated from the previously mentioned devices and have the potential to gain a high level of information about the structural make-up and behavioural patterns of a single cell or network of cells as an external influence is applied to them. Initially, identifying rare cells by microscopy was highly laborious with the accuracy and sensitivity being a subject of the fatigue encountered by the viewer. However, throughput and accuracy were improved as the newly introduced digital camera began to rise in popularity in the late 80s/early 90s (Mansi et al. 1988, Lee et al. 1989, Mesker et al. 1994) and were incorporated into the first digital image microscopy systems. Since then, newer systems have been developed with the aim being to increase the resolution and speed and therefore acquire more detailed information about the cell (Kraeft et al. 2004, Krivacic et al. 2004, Hsieh et al. 2006). However, the biggest advancement of this technique came with the incorporation of sensors that monitor the *xy* position of the slide on the computer-controlled motorised microscope stage, which moves at 0.5 μm-steps per each laser scan, perpendicular to the scan (Pozarowski et al. 2006). This enables the detected cells of interest (either by scattered laser light or specimen-emitted fluorescence) to be relocalised in sequential measurements. This ability to get temporal information has been used to 1) discriminate, through cell morphology, between the genuine apoptotic cells and 'false-positive' cells in peripheral blood and bone marrow of leukemic patients undergoing chemotherapy (Bedner et al. 1999), 2) reveal translocation of proteins throughout the cell during mitosis (Kakino et al. 1996), 3) measure kinetic reactions within individual cells in large populations

(Bedner et al. 1998), and 4) enumerate cells at the completion of therapy to determine the likelihood of early relapse (Pachmann et al. 2008).

Single-cell analysis techniques have already shown promise to reveal important information about the behaviour of cells within the human body and will continue to reveal further insights as spatial and temporal resolution is improved with later generations of analytical assays and instruments.

Conclusions and Future Perspectives

Several methods for single-cell isolation have already been developed, and by combining these isolation techniques with newly developed single-cell assays, a focus has been directed towards solving clinical problems. The key to gaining more valuable clinical information of this nature lies within the development of single-cell isolation strategies with a higher degree of control over which single cell to analyse. The shift in focus down to one individual cell has led towards a better ability to understand cellular heterogeneity. To gain a better understanding of important events within the human body will require the cells that are responsible for these events to be determined prior to their analysis. For this reason, the next generation of single-cell isolation strategies are required to incorporate a selective element to discriminate between different types of individual cells.

References

Ahn, K., Kerbage, C., Hunt, T. P., Westervelt, R. M., Link, D. R. & Weitz, D. A. (2006) Dielectrophoretic manipulation of drops for high-speed microfluidic sorting devices, *Applied Physics Letters*, 88, 2.

Andersson Svahn, H. & van den Berg, A. (2007) Single cells or large populations?, *Lab on a Chip*, 7, 5, 544-546.

Bedner, E., Li, X., Gorczyca, W., Melamed, M. R. & Darzynkiewicz, Z. (1999) Analysis of apoptosis by laser scanning cytometry, *Cytometry*, 35, 3, 181-195.

Bedner, E., Melamed, M. R. & Darzynkiewicz, Z. (1998) Enzyme kinetic reactions and fluorochrome uptake rates measured in individual cells by laser scanning cytometry, *Cytometry*, 33, 1, 1-9.

Benavente-Babace, A., Gallego-Pérez, D., Hansford, D. J., Arana, S., Pérez-Lorenzo, E. & Mujika, M. (2014) Single-cell trapping and selective treatment via co-flow within a microfluidic platform, *Biosensors & Bioelectronics*, 61, 0, 298-305.

Bielas, J. H., Loeb, K. R., Rubin, B. P., True, L. D. & Loeb, L. A. (2006) Human cancers express a mutator phenotype, *Proceedings of the National Academy of Sciences of the United States of America*, 103, 48, 18238-18242.

Bielas, J. H. & Loeb, L. A. (2005) Mutator phenotype in cancer: Timing and perspectives, *Environmental and Molecular Mutagenesis*, 45, 2-3, 206-213.

Brouzes, E., Medkova, M., Savenelli, N., Marran, D., Twardowski, M., Hutchison, J. B., Rothberg, J. M., Link, D. R., Perrimon, N. & Samuels, M. L. (2009) Droplet microfluidic technology for single-cell high-throughput screening, *Proceedings of the National Academy of Sciences of the United States of America*, 106, 34, 14195-14200.

Chabert, M., Dorfman, K. D. & Viovy, J. L. (2005) Droplet fusion by alternating current (AC) field electrocoalescence in microchannels, *Electrophoresis*, 26, 19, 3706-3715.

Chen, Y.-C., Cheng, Y.-H., Kim, H. S., Ingram, P. N., Nor, J. E. & Yoon, E. (2014) Paired single cell co-culture microenvironments isolated by two-phase flow with continuous nutrient renewal, *Lab on a Chip*, 14, 16, 2941-2947.

Chin, V. I., Taupin, P., Sanga, S., Scheel, J., Gage, F. H. & Bhatia, S. N. (2004) Microfabricated platform for studying stem cell fates, *Biotechnology and Bioengineering*, 88, 3, 399-415.

Clausell-Tormos, J., Lieber, D., Baret, J.-C., El-Harrak, A., Miller, O. J., Frenz, L., Blouwolff, J., Humphry, K. J., Koster, S., Duan, H., Holtze, C., Weitz, D. A., Griffiths, A. D. & Merten, C. A. (2008) Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms, *Chemistry & Biology*, 15, 5, 427-437.

Cooper, G. M. & Hausman, R. E. (2007) *The Cell*, ASM Press, Washington, DC.

Deutsch, M., Deutsch, A., Shirihai, O., Hurevich, I., Afrimzon, E., Shafran, Y. & Zurgil, N. (2006) A novel miniature cell retainer for correlative high-content analysis of individual untethered non-adherent cells, *Lab on a Chip*, 6, 8, 995-1000.

Di Carlo, D., Aghdam, N. & Lee, L. P. (2006a) Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays, *Analytical Chemistry*, 78, 14, 4925-4930.

Di Carlo, D. & Lee, L. P. (2006) Dynamic single-cell analysis for quantitative biology, *Analytical Chemistry*, 78, 23, 7918-7925.

Di Carlo, D., Wu, L. Y. & Lee, L. P. (2006b) Dynamic single cell culture array, *Lab on a Chip*, 6, 11, 1445-1449.

Edd, J. F., Di Carlo, D., Humphry, K. J., Koster, S., Irimia, D., Weitz, D. A. & Toner, M. (2008) Controlled encapsulation of single-cells into monodisperse picolitre drops, *Lab on a Chip*, 8, 8, 1262-1264.

Gach, P. C., Wang, Y., Phillips, C., Sims, C. E. & Allbritton, N. L. (2011) Isolation and manipulation of living adherent cells by micromolded magnetic rafts, *Biomicrofluidics*, 5, 3.

Hashimshony, T., Wagner, F., Sher, N. & Yanai, I. (2012) CEL-Seq: Single-cell RNA-seq by multiplexed linear amplification, *Cell Reports*, 2, 3, 666-673.

He, M. Y., Edgar, J. S., Jeffries, G. D. M., Lorenz, R. M., Shelby, J. P. & Chiu, D. T. (2005) Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets, *Analytical Chemistry*, 77, 6, 1539-1544.

Heitzer, E., Auer, M., Gasch, C., Pichler, M., Ulz, P., Hoffmann, E. M., Lax, S., Waldispuehl-Geigl, J., Mauermann, O., Lackner, C., Hoefler, G., Eisner, F., Sill, H., Samonigg, H., Pantel, K., Riethdorf, S., Bauernhofer, T., Geigl, J. B. & Speicher, M. R. (2013) Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing, *Cancer Research*, 73, 10, 2965-2975.

Hsieh, H. B., Marrinucci, D., Bethel, K., Curry, D. N., Humphrey, M., Krivacic, R. T., Kroener, J., Kroener, L., Ladanyi, A., Lazarus, N., Kuhn, P., Bruce, R. H. & Nieva, J. (2006) High speed detection of circulating tumor cells, *Biosensors & Bioelectronics*, 21, 10, 1893-1899.

Islam, S., Kjallquist, U., Moliner, A., Zajac, P., Fan, J.-B., Lonnerberg, P. & Linnarsson, S. (2011) Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq, *Genome Research*, 21, 7, 1160-1167.

Joensson, H. N., Samuels, M. L., Brouzes, E. R., Medkova, M., Uhlen, M., Link, D. R. & Andersson-Svahn, H. (2009) Detection and analysis of low-abundance cell-surface biomarkers using enzymatic amplification in microfluidic droplets, *Angewandte Chemie-International Edition*, 48, 14, 2518-2521.

Joensson, H. N. & Svahn, H. A. (2012) Droplet Microfluidics-A Tool for Single-Cell Analysis, *Angewandte Chemie-International Edition*, 51, 49, 12176-12192.

Kakino, S., Sasaki, K., Kurose, A. & Ito, H. (1996) Intracellular localization of cyclin B1 during the cell cycle in glioma cells, *Cytometry*, 24, 1, 49-54.

Koester, S., Angile, F. E., Duan, H., Agresti, J. J., Wintner, A., Schmitz, C., Rowat, A. C., Merten, C. A., Pisignano, D., Griffiths, A. D. & Weitz, D. A. (2008) Drop-based microfluidic devices for encapsulation of single cells, *Lab on a Chip*, 8, 7, 1110-1115.

Kraeft, S. K., Ladanyi, A., Galiger, K., Herlitz, A., Sher, A. C., Bergsrud, D. E., Even, G.,

Brunelle, S., Harris, L., Salgia, R., Dahl, T., Kesterson, J. & Chen, L. B. (2004) Reliable and sensitive identification of occult tumor cells using the improved rare event Imaging system, *Clinical Cancer Research*, 10, 9, 3020-3028.

Krivacic, R. T., Ladanyi, A., Curry, D. N., Hsieh, H. B., Kuhn, P., Bergsrud, D. E., Kepros, J. F., Barbera, T., Ho, M. Y., Chen, L. B., Lerner, R. A. & Bruce, R. H. (2004) A rare-cell detector for cancer, *Proceedings of the National Academy of Sciences of the United States of America*, 101, 29, 10501-10504.

Lee, B. R., Haseman, D. B. & Reynolds, C. P. (1989) A digital image microscopy system for rare-event detection using fluorescent-probes, *Cytometry*, 10, 3, 256-262.

Lindstrom, S. & Andersson-Svahn, H. (2011) Miniaturization of biological assays - Overview on microwell devices for single-cell analyses, *Biochimica Et Biophysica Acta-General Subjects*, 1810, 3, 308-316.

Lindstrom, S., Eriksson, M., Vazin, T., Sandberg, J., Lundeberg, J., Frisen, J. & Andersson-Svahn, H. (2009) High-density microwell chip for culture and analysis of stem cells, *Plos One*, 4, 9.

Link, D. R., Anna, S. L., Weitz, D. A. & Stone, H. A. (2004) Geometrically mediated breakup of drops in microfluidic devices, *Physical Review Letters*, 92, 5.

Ma, Z., Lee, R. W., Li, B., Kenney, P., Wang, Y., Erikson, J., Goyal, S. & Lao, K. (2013) Isothermal amplification method for next-generation sequencing, *Proceedings of the National Academy of Sciences of the United States of America*, 110, 35, 14320-14323.

Mansi, J. L., Mesker, W. E., McDonnell, T., Vandrielkulker, A. M. J., Ploem, J. S. & Coombes, R. C. (1988) Automated screening for micrometasteses in bone marrow smears, *Journal of Immunological Methods*, 112, 1, 105-111.

Mesker, W. E., Vanderburg, M. J. M., Oud, P. S., Knepfle, C., Ouwerkerkvonvelzen, M. C. M., Schipper, N. W. & Tanke, H. J. (1994) Detection of immunocytochemically stained rare events using image-analysis, *Cytometry*, 17, 3, 209-215.

Navin, N., Kendall, J., Troge, J., Andrews, P., Rodgers, L., McIndoo, J., Cook, K., Stepansky, A., Levy, D., Esposito, D., Muthuswamy, L., Krasnitz, A., McCombie, W. R., Hicks, J. & Wigler, M. (2011) Tumour evolution inferred by single-cell sequencing, *Nature*, 472, 7341, 90-U119.

Norris, J. R. & Ribbons, D. W. (2006) *Methods in Microbiology*, Academic Press, London, 1-480.

Ostuni, E., Chen, C. S., Ingber, D. E. & Whitesides, G. M. (2001) Selective deposition of proteins and cells in arrays of microwells, *Langmuir*, 17, 9, 2828-2834.

Pachmann, K., Dengler, R., Lobodasch, K., Froehlich, F., Kroll, T., Rengsberger, M., Schubert, R. & Pachmann, U. (2008) An increase in cell number at completion of therapy may develop as an indicator of early relapse, *Journal of Cancer Research and Clinical Oncology*, 134, 1, 59-65.

Powell, A. A., Talasaz, A. H., Zhang, H. Y., Coram, M. A., Reddy, A., Deng, G., Telli, M. L., Advani, R. H., Carlson, R. W., Mollick, J. A., Sheth, S., Kurian, A. W., Ford, J. M., Stockdale, F. E., Quake, S. R., Pease, R. F., Mindrinos, M. N., Bhanot, G., Dairkee, S. H., Davis, R. W. & Jeffrey, S. S. (2012) Single cell profiling of circulating tumor cells: Transcriptional heterogeneity and diversity from breast cancer cell lines, *Plos One*, 7, 5.

Pozarowski, P., Holden, E. & Darzynkiewicz, Z. (2006) Laser scanning cytometry: Principles and applications, *Methods in Molecular Biology*, 319, 165-192.

Ramskold, D., Luo, S., Wang, Y.-C., Li, R., Deng, Q., Faridani, O. R., Daniels, G. A., Khrebtukova, I., Loring, J. F., Laurent, L. C., Schroth, G. P. & Sandberg, R. (2012) Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells, *Nature Biotechnology*, 30, 8, 777-782.

Rettig, J. R. & Folch, A. (2005) Large-scale single-cell trapping and imaging using microwell arrays, *Analytical Chemistry*, 77, 17, 5628-5634.

Revzin, A., Sekine, K., Sin, A., Tompkins, R. G. & Toner, M. (2005) Development of a microfabricated cytometry platform for

characterization and sorting of individual leukocytes, *Lab on a Chip*, 5, 1, 30-37.

Sandberg, R. (2014) Entering the era of single-cell transcriptomics in biology and medicine, *Nature Methods*, 11, 1, 22-24.

Sasagawa, Y., Nikaido, I., Hayashi, T., Danno, H., Uno, K. D., Imai, T. & Ueda, H. R. (2013) Quartz-Seq: a highly reproducible and sensitive single-cell RNA sequencing method, reveals non-genetic gene-expression heterogeneity, *Genome Biology*, 14, 4.

Skelley, A. M., Kirak, O., Suh, H., Jaenisch, R. & Voldman, J. (2009) Microfluidic control of cell pairing and fusion, *Nature Methods*, 6, 2, 147-152.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B. B., Siddiqui, A., Lao, K. & Surani, M. A. (2009) mRNA-Seq whole-transcriptome analysis of a single cell, *Nature Methods*, 6, 5, 377-U86.

Tang, F., Lao, K. & Surani, M. A. (2011) Development and applications of single-cell transcriptome analysis, *Nature Methods*, 8, 4, S6-S11.

Taylor, L. C. & Walt, D. R. (2000) Application of high-density optical microwell arrays in a live-cell biosensing system, *Analytical Biochemistry*, 278, 2, 132-142.

Tokimitsu, Y., Kishi, H., Kondo, S., Honda, R., Tajiri, K., Motoki, K., Ozawa, T., Kadowaki, S., Obata, T., Fujiki, S., Tateno, C., Takaishi, H., Chayama, K., Yoshizato, K., Tamiya, E., Sugiyama, T. & Muraguchi, A. (2007) Single lymphocyte analysis with a microwell array chip, *Cytometry Part A*, 71A, 12, 1003-1010.

Walling, M. A. & Shepard, J. R. E. (2011) Cellular heterogeneity and live cell arrays, *Chemical Society Reviews*, 40, 7, 4049-4076.

Wang, Y., Phillips, C., Xu, W., Pai, J.-H., Dhopeshwarkar, R., Sims, C. E. & Allbritton, N. (2010) Micromolded arrays for separation of adherent cells, *Lab on a Chip*, 10, 21, 2917-2924.

Wang, Y., Waters, J., Leung, M. L., Unruh, A., Roh, W., Shi, X., Chen, K., Scheet, P., Vattathil, S., Liang, H., Multani, A., Zhang, H., Zhao, R., Michor, F., Meric-Bernstam, F. & Navin, N. E. (2014) Clonal evolution in breast cancer revealed by single nucleus genome sequencing, *Nature*, 512, 7513, 155-160.

Wood, D. K., Weingeist, D. M., Bhatia, S. N. & Engelward, B. P. (2010) Single cell trapping and DNA damage analysis using microwell arrays, *Proceedings of the National Academy of Sciences of the United States of America*, 107, 22, 10008-10013.

Zhang, K., Han, X., Li, Y., Li, S. Y., Zu, Y., Wang, Z. & Qin, L. (2014) Hand-held and integrated single-cell pipettes, *Journal of the American Chemical Society*, 136, 31, 10858-10861.

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Can Small Islands Tell Large(r) Stories? The Microcosm of Nepean Island, Norfolk Island Archipelago

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Abstract

Norfolk Island, South Pacific, provides linguists a near laboratory case study in naming, language contact, and environmental management. The two languages spoken on the island, Norf'k – the language of the descendants of the Pitcairn Islanders – and English, are both used in place-naming. This short note analyses the toponyms of Nepean Island, a small uninhabited island 800 metres south of Norfolk. It questions whether Nepean is a microcosm of naming behaviour for the rest of the Norfolk macrocosm. For its size, Nepean contains a large number of toponyms. The paper suggests the uninhabited nature of Nepean may have resulted in fewer commemorative anthroponymic toponyms, a situation unlike naming patterns in the rest of the archipelago. Nepean offers a study of naming a small no-man's land as compared to naming a larger occupied land.

Nepean Island

Norfolk Island (29°02'S, 167°57'E), a remote isolated island archipelago and external territory of Australia in the southwest Pacific Ocean 1700 kilometres east of the Australian mainland and 1100 kilometres from Auckland, provides toponymists and linguists a near laboratory case study in naming, toponymy, and language change and contact. It has a permanent population of around 2000. About half of this population are descendants of the *Bounty* mutineers who were moved from Pitcairn Island to Norfolk in 1856. The archipelago consists of three major islands and several nearby offshore rocky outcrops. The three islands in the archipelago run from north to south: Norfolk (35 km^2) is the largest, and two smaller uninhabited islands are Nepean (1 km^2) and Phillip (5 km^2) (Figure 1).

What makes Norfolk Island attractive for linguists is its historically diglossic language situation; Norf'k – the language of the descendants of the *Bounty* mutineers and their Tahitian wives – and English, are both spoken on the island and both are used in place-naming. This short note considers the toponyms of Nepean Island, a small uninhabited island 800 metres to the south of Kingston, Norfolk's administrative centre, and questions whether Nepean is a microcosm of naming behaviour representative of the rest of the Norfolk macrocosm. The question posed is whether a small island can tell a larger story of place-naming processes. The results of human habitation on these processes are additionally assessed.

Nepean Island has a large number of toponyms for its size. Its toponyms represent a microtoponymic case study which may be

representative of toponymy on the Norfolk Archipelago as a whole, because the island features a large number of culturally important names within a relatively small area. Despite its small size, Nepean is an important element in the Norfolk landscape. Its grassy, craggy topography is clearly visible from most vantage points on the southern region of the larger Norfolk (Figure 2). The 200 Norfolk Island pines which used to cover Nepean were cleared long before the Pitcairners arrived in 1856. The physical makeup of the island bears scars from the first two penal settlements, particularly the Second Settlement (1825-1855), when sandstone quarrying resulted in the well-known area and placename The Convict Steps (Em Steps in Norf'k) on the eastern side of the island. Nepean has a large population of sea birds, and the Norfolk community uses the island for activities like fishing, camping, and collecting eggs of the whale bird, the sooty tern (*sterna fuscata*), a common sea bird which nests predominantly on Nepean and Phillip.

Aside from research into the natural history of Nepean (see references to Nepean Island in Endersby 2003), management plans for the inclusion of Nepean as a public reserve (Norfolk Island Parks & Forestry Service 2003), a small sketchy map in Coleman (1991: 4), and a few comments on Nepean toponyms in a rambler's (hiker's) guide to Norfolk (Hoare 1994), there has not been a detailed toponymic survey of this small, uninhabited island.

Methods

Nepean toponyms were collected on three field trips on Norfolk Island between 2008 and 2009. Approximately five informal interviews with members of the community were conducted and subsequent follow up questionnaires based on a more precise list of placenames derived from archival research and the initial interviews were carried out (a more comprehensive list of Norfolk toponyms is published in Nash (2013). Table 1 presents a list of Nepean placenames with their history. Figure 3 plots the location of these names.

Figure 1: The Norfolk Archipelago.

Figure 2: Nepean Island topography.

The Data

Table 1: Nepean Island toponyms and histories.

Name	History
East End	The easternmost point of the island.
Mary Hamilton Reef/Rocks	An eponymous placename doublet[1] remembering the reef and rocks where the steam liner *Mary Hamilton* came aground in the early 1900s. It is also the name of a diving site in the same area along with other nearby sites Blues Cathedral and Black Coral.
Poison Bay/Pizen Bay	The location and etymology of this name are questionable. Reliable sources place it on the northern coast, while the *Nepean Island – Plan of Management* (Norfolk Island Parks & Forestry Service. 2003:1) places it in the location where the placename Up ar Sand appears on the map opposite. It is either named after the 'poison wind' which comes from the north-east across Norfolk during inclement weather and can burn crops, or because of the local Norfolk 'poison weed' which may have been found on Nepean. Pizen Bay is a suspected secondary name attributed to a gentleman by the name of Pizen who supposedly used to fish here.
The Convict Steps / Em Steps	The English and Norf'k placename doublet for the convict steps which were created when stone was mined by convicts for constructing buildings in Norfolk's administrative centre in Kingston. Also known as Dem Steps or Em Steps. Although mooring on Nepean is difficult, this is an easy place to gain access to dry land by boat due to the flat rocks at sea level near The Convict Steps.
The Crack, Crack	A location on the far west of Nepean which is a favourite rock fishing site. At low tide one can easily cross from The Crack to West End but during high tide one has to jump because the higher swells submerge the rock passage. This name can take the Norf'k definite article 'ar' to form Ar Crack.
The Saddle, Saddle	Descriptive name for the undulating topography which appears like a saddle from one side, especially from Queen Elizabeth Lookout (also known as Lizzies), a turn in the Rooty Hill Road on Norfolk which looks out to Nepean. This name can take the Norf'k definite article 'ar' to form Ar Saddle.

[1] A toponym doublet is where two different versions of one name exist for the same place.

The Skull, Skull	A landscape feature on the extreme south of Nepean, which when the sun shines on it looks like a skull. This name can take the Norf'k definite article 'ar' to form Ar Skull.
The Stump, Stump	Named after the remains of a Norfolk pine which used to be located in the narrow southern portion of Nepean. The Stump was used in locating several offshore fishing grounds, which were lost once The Stump was pushed over. This name can take the Norf'k definite article 'ar' to form Ar Stump.
Under Stump	The rocky area at sea level under where The Stump used to stand.
Unicorn	Located between The Stump and The Skull, Unicorn is another pointed landscape feature which has been described as looking like a unicorn.
Up ar Sand	Translated as 'up on the sand' or 'up on the beach' in English, this is the only sandy area of significant size on Nepean. Although most likely incorrect, several informants have proposed Poison Bay is located here.
West End	The westernmost point of the island.

Like the larger corpus of Norfolk data presented in Nash (2013), the Nepean microcosm presents some generalities that apply to Norfolk toponymy proper:

Figure 3: Nepean Island land-based toponyms

1. Norfolk toponyms (e.g. Fata Fata) and Nepean toponyms contain Norf'k lexemes.
2. Toponym doublets (e.g. Poison Bay/Pizen Bay) are present on Norfolk (e.g. Kingston/Down-a-Town).
3. Descriptive names are common on Nepean as on Norfolk.
4. Two commemorative names (e.g. Mary Hamilton Reef/Rocks) exist on Nepean as they do on Norfolk (e.g. Johnnies Stone). However, Nepean commemorative names are not named after Norfolk Islanders (Pitcairn descendants) as they are on larger Norfolk.
5. Non-proper monolexemes (i.e. single words, in this case nouns with or without articles) are productive as toponyms on Nepean (e.g. Skull, The Stump) and on

Norfolk (e.g. Cascade). However, monolexemes on Norfolk are typically commemorative (e.g. Monty) whereas there are no commemorative monolexeme toponyms on Nepean.

6. The microtoponymy of Nepean illustrates how the locations, histories, and language of placenames become obscured. For example, by analysing the linguistic form and cultural history of a toponym doublet such as Poison Bay/Pizen Bay, it is unclear whether either or both names are English or Norf'k.

Small Islands, Larger Stories?

On returning to the question of whether small islands can tell large(r) toponymic stories, there is a key difference between Nepean and Norfolk monolexemes: Norfolk monolexemes (e.g. Monty, Barnaby, Avalon) are often commemorative where Nepean's, being largely descriptive, are not (e.g. Stump, Unicorn). This difference could possibly be attributed to the fact that Nepean is uninhabited whereas Norfolk is inhabited. As a microcosm, Nepean toponymy illustrates an incomplete version of Norfolk toponymy; Nepean and Norfolk are different, most strikingly in terms of how human habitation is represented in place-naming. Nepean's offering of a study of naming a small 'no-man's land' as compared to the naming of the larger 'occupied land' of Norfolk reveals significant differences.

The microtoponymy of Nepean Island, an island uninhabitable due to the lack of running water and shelter, provides insight into what tools humans use to utilise, understand, and describe a small and yet well-visited and historically significant geographical area. This short note has documented and mapped Nepean placenames and presented a resource which other toponymists and linguists can use in future research into small island toponymies.

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References

Coleman, N. (1991) *The Nature of Norfolk Island*, Sea Resource Centre, Rochedale South, Queensland.

Endersby, I. (2003) *Bibliography of Norfolk Island Natural History: Bishop Museum occasional papers no. 7*, Bishop Museum Press, Honolulu.

Green, T. H. (1973) Petrology and Geochemistry of Basalts from Norfolk Island, *Journal of the Geological Society of Australia* 20(3), 259-272.

Hoare, M. (1994) *Rambler's Guide to Norfolk Island*, Photopress International, Norfolk Island.

Nash, J. (2013) *Insular Toponymies: Place-naming on Norfolk Island, South Pacific and Dudley Peninsula, Kangaroo Island*, John Benjamins, Amsterdam/Philadelphia.

Norfolk Island Parks and Forestry Service (2003) *Nepean Island–Plan of Management*, Norfolk Island Legislative Assembly, Norfolk Island.

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Thesis Abstract

Investigations of the Acoustics of the Vocal Tract and Vocal Folds *in vivo*, *ex vivo* and *in vitro*

Noel Nagy Nabih Hanna

Abstract of a thesis for a Doctorate of Philosophy
submitted to
The University of New South Wales, Australia
and the
Université de Grenoble-Alpes, France

The acoustic impedance of the vocal tract filter was measured in vivo from 10 to 4200 Hz with the glottis closed and during phonation. Frequencies, magnitudes and bandwidths were measured for the acoustic and for the mechanical resonances of the surrounding tissues. The energy losses in the vocal tract were five-fold higher than the visco-thermal losses of a dry, smooth rigid cylinder, and increase during phonation. Using a simple vocal tract model and measurements during inhalation, the resonances of the subglottal system were also estimated.

In a separate experiment excised larynges were used to investigate the control of the voice fundamental frequency by either air supply or mechanical control. All else equal, and excluding the discontinuities and hysteresis observed, the fundamental frequency was approximately proportional to the square root of subglottal pressure. Additionally, airflow through the glottis caused a narrowing of the aryepiglottic larynx in the absence of muscular control.

Finally, possible effects of the filter on the source were demonstrated using a water-filled latex vocal fold replica: changing the aero-acoustic load of the model tract by inserting a straw at the model lips, a technique used in speech therapy, changed the fundamental frequency.

http://handle.unsw.edu.au/1959.4/54304

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Awards 2016

The following awards are offered by the Royal Society in 2016. Please see the specific page for details of each award.

Award	Eligibility		Closing date
Clarke Medal	Field: Seniority: Work considered: Location of work: Application by:	Zoology Any "Significant contribution" Australia Nomination[1]	30th September, 2016
Edgeworth David Medal	Field: Seniority: Work considered: Location of work: Application by:	Any < 35 "Distinguished contribution" Australia Nomination	30th September, 2016
James Cook Medal	Field: Seniority: Work considered: Location of work: Application by:	"Science & human welfare" Any "Outstanding contribution" Southern Hemisphere Nomination	30th September, 2016
Warren Prize	Field: Seniority: Work considered: Location of work: Application by:	Engineering or technology In professional practice "of national or international significance" NSW Paper submitted to Journal	30th September, 2016
History and Philosophy of Science Medal	Field: Seniority: Work considered: Location of work: Application by:	History and philosophy of science Any "significant contribution to the understanding of the history and philosophy of science" Any Nomination or direct submission	30th September, 2016
RSNSW Scholarships Jak Kelly Award	Field: Seniority: Work considered: Location of work: Application by:	Any Enrolled research student on 1 July Research project NSW or ACT Application by student/ endorsed by supervisor	30th September, 2016

[1] Nomination by a senior member of the nominee's organisation (for example Dean, Pro Vice Chancellor of a university, Section or Division Head of CSIRO), or a member of the Royal Society of New South Wales.

Clarke Medal 2016

The Clarke Medal was established to acknowledge the contribution by Rev William Branwhite Clarke MA FRS FGS, Vice-President of the Royal Society of New South Wales from 1866 to 1878. The Medal is awarded annually for distinguished work in the natural sciences of geology, botany and zoology done in Australia and its Territories.

The Medal is awarded by rotation in the fields of geology, botany and zoology. This year's award is in the field of Zoology in all its aspects, and nominations are called for the names of suitable persons who have contributed significantly to this science.

The Council requests that every nomination should be accompanied by a list of publications, a full *curriculum vitae*, and also by a statement clearly indicating which part of the nominee's work was done in Australia and which part was done overseas. Agreement of the nominee must be obtained by the nominator before submission and included with the nomination.

The winner will be expected to write a review paper of their work for submission to the Society's Journal and Proceedings. In cases where the Council of the Society is unable to distinguish between two persons of equal merit, preference will be given to a Member of the Society.

Nominations and supporting material should be submitted by email (secretary@royalsoc.org.au) to the Royal Society of New South Wales marked to the attention of the Honorary Secretary, not later than 30th September 2016.

The winner will be announced and the Medal presented at the Annual Dinner of the Royal Society usually held in April in the year following the award. The winner will be notified in December.

Edgeworth David Medal 2016

The Edgeworth David Medal, established in memory of Professor Sir Tannatt William Edgeworth David FRS, a past President of the Society, is awarded for distinguished contributions by a young scientist.

The conditions of the award of the Medal are:

- The recipient must be under the age of 35 years at 1 January 2016.
- The Medal will be for work done mainly in Australia or its Territories or contributing to the advancement of Australian science.

Nominations are called for the names of suitable persons who have contributed significantly to science, including scientific aspects of agriculture, engineering, dentistry, medicine and veterinary science.

The Council requests that every nomination should be accompanied by a list of publications, a full *curriculum vitae*, and also by a statement clearly indicating which part of the nominee's work was done in Australia and which part was done overseas. Agreement of the nominee must be obtained by the nominator before submission and included with the nomination.

The winner will be expected to write a review paper of their work for submission to the Society's Journal and Proceedings. In cases where the Council of the Society is unable to distinguish between two persons of equal merit, preference will be given to a Member of the Society.

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James Cook Medal 2016

The James Cook Medal is awarded at intervals for outstanding contributions to science and human welfare in and for the Southern Hemisphere.

The James Cook Medal was established in 1947 with funding by Henry Ferdinand Halloran. Halloran, who had joined the Society in 1892 as a 23 year-old, was a surveyor, engineer and town planner. He did not publish anything in the Society's Journal, but he was a very enthusiastic supporter of research. Halloran funded what were to become the Society's two most prestigious awards, the James Cook Medal and the Edgeworth David Medal, the latter medal being for young scientists.

The Council requests that every nomination should be accompanied by a list of publications, a full *curriculum vitae*, and also by a statement clearly indicating which part of the nominee's work was done in Australia and which part was done overseas. Agreement of the nominee must be obtained by the nominator before submission and included with the nomination.

The winner will be expected to write a review paper of their work for submission to the Society's Journal and Proceedings. In cases where the Council of the Society is unable to distinguish between two persons of equal merit, preference will be given to a Member of the Society.

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The winner will be announced and the Medal presented at the Annual Dinner of the Royal Society usually held in April in the year following the award. The winner will be notified in December.

Warren Prize 2016

William Henry Warren established the first faculty of engineering in New South Wales and was appointed as its Professor at the University of Sydney in 1884. Professor Warren was President of the Royal Society of New South Wales on two occasions. He had a long career of more than 40 years and during this time was considered to be the most eminent engineer in Australia. When the Institution of Engineers, Australia was established in 1919, Professor Warren was elected as its first President. He established an internationally respected reputation for the Faculty of Engineering at the University of Sydney and published extensively, with many of his papers being published in the Journal and Proceedings of the Royal Society of New South Wales.

The Warren Prize has been established by the Royal Society of NSW to acknowledge Professor Warren's contribution both to the Society and to the technological disciplines in Australia and internationally. The aim of the award is to recognise research of national or international significance by engineers and technologists in their professional practice. The research must have originated or have been carried out principally in New South Wales. The prize is $500.

Entries are by submission of an original paper which reviews the research field, highlighting the contributions of the candidate, and identifying its national or international significance. Preference will be given to entries that demonstrate relevance across the spectrum of knowledge – science, art, literature and philosophy – that the Society promotes.

The winning paper and a selection of other entries submitted will be peer-reviewed and are expected to be published in the Journal and Proceedings of the Royal Society of New South Wales. Depending on the number of acceptable entries, there may be a special edition of the Journal and Proceedings that would be intended to showcase research by early- and mid-career Australian researchers.

The paper should be submitted by email (secretary@royalsoc.org.au) to the Royal Society of New South Wales marked to the attention of the Honorary Secretary, not later than 30th September 2016. The manuscript will be passed on to the Editor of the Journal for peer review.

The winner will be announced and the Medal presented at the Annual Dinner of the Royal Society usually held in April in the year following the award. The winner will be notified in December.

History and Philosophy of Science Medal 2016

The Royal Society of NSW History and Philosophy of Science Prize was established in 2014 to recognise outstanding achievement in the History and Philosophy of Science, and the inaugural award was made to Ann Moyal in 2015. It is anticipated that this Prize, like the Society's other awards, will become one of the most prestigious awards offered in Australia in this field. The winner will be awarded a medal.

Persons nominated will have made a significant contribution to the understanding of the history and philosophy of science, with preference being given to the study of ideas, institutions and individuals of significance to the practice of the natural sciences in Australia.

Entries may be made by nomination or direct submission. All entries should be accompanied by a full *curriculum vitae* and include a one-page statement setting out the case for award. In the case of nominations, the agreement of the nominee must be obtained by the nominator before submission and included with the entry.

The winner will be expected to submit an unpublished essay, drawing on recent work, which will be considered for publication in the Society's Journal and Proceedings during the following year.

Nominations and supporting material should be submitted by email (secretary@royalsoc.org.au) to the Royal Society of New South Wales marked to the attention of the Honorary Secretary, not later than 30th September 2016.

The winner will be announced and the Medal presented at the Annual Dinner of the Royal Society usually held in April in the year following the award. The winner will be notified in December.

Royal Society of NSW Scholarships 2016

The Royal Society of New South Wales is the oldest learned society in Australia, tracing its origins to 1821. It has a long tradition of encouraging and supporting scientific research and leading intellectual life in the State. The Council of the Society funds the Royal Society of New South Wales Scholarships in order to acknowledge outstanding achievements by early-career individuals working towards a research degree in a science-related field.

Applications for Royal Society of New South Wales Scholarships are sought from candidates working in a science-related field within New South Wales or the Australian Capital Territory. There is no restriction with respect to field of study and up to three Scholarships will be awarded each year. Applicants must be Australian citizens or Permanent Residents of Australia. Applicants must be enrolled as research students at a university in NSW or the ACT on 1st July in the year of the award.

Applications for a RSNSW Scholarship must include:

- 500-word summary of the work.
- Statement of the significance of the work, particularly within the broader context of your chosen field.
- *Curriculum vitae*, including details of their research candidacy.
- Letter of support from your research supervisor.

Applications should be submitted by email (secretary@royalsoc.org.au) to the Royal Society of New South Wales marked to the attention of the Honorary Secretary, not later than 30th September 2016.

Jak Kelly Award 2016

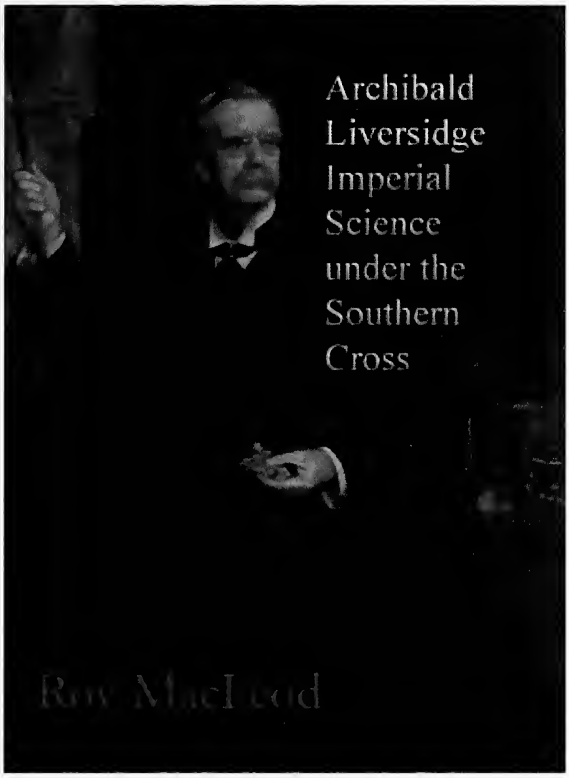
The Jak Kelly Award is awarded jointly with the Australian Institute of Physics to the best PhD student talk presented at a joint meeting with the AIP.

The award consists of an engraved plaque, a $500 prize and a year of membership of the Society. The successful applicants will present their work to a meeting of the Royal Society in 2017, and will be asked to prepare a paper for the Society's Journal and Proceedings.

The winners of both awards will be notified in December.

Archibald Liversidge:
Imperial Science under the Southern Cross

Roy MacLeod
Royal Society of New South Wales, in association with Sydney University Press
ISBN 9781-9208-9880-9



When Archibald Liversidge first arrived at the University of Sydney in 1872 as Reader in Geology and Assistant in the Laboratory, he had about ten students and two rooms in the main building. In 1874, he became Professor of Geology and Mineralogy and by 1879 he had persuaded the University Senate to open a Faculty of Science. He became its first Dean in 1882.

In 1880, he visited Europe as a trustee of the Australian Museum and his report helped to establish the Industrial, Technological and Sanitary Museum which formed the basis of the present Powerhouse Museum's collection. Liversidge also played a major role in establishing the *Australasian Association for the Advancement of Science* which held its first congress in 1888.

This book is essential reading for those interested in the development of science in colonial Australia, particularly the fields of crystallography, mineral chemistry, chemical geology and strategic minerals policy.

To order your copy, please complete the Liversidge Book Order Form available at: http://royalsoc.org.au/publications/books/McLeod_Liversidge_Order_Form.pdf and return it together with your payment to:

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(Liversidge Book),
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The Royal Society of New South Wales

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The templates available on the Journal website should be used for preparing manuscripts. Full instructions for preparing submissions are also given on the website.

If the file-size is too large to email it should be placed on a CD-ROM or other digital media and posted to:

The Honorary Secretary (Editorial),
The Royal Society of New South Wales,
PO Box 576,
Crows Nest, NSW 1585
Australia

Manuscripts will be reviewed by the Editor, in consultation with the Editorial Board, to decide whether the paper will be considered for publication in the Journal. Manuscripts are subjected to peer review by at least one independent reviewer. In the event of initial rejection, manuscripts may be sent to other reviewers.

Papers (other than those specially invited by the Editorial Board) will only be considered if the content is either substantially new material that has not been published previously, or is a review of a major research programme. Papers presenting aspects of the historical record of research carried out within Australia are particularly encouraged. In the case of papers presenting new research, the author must certify that the material has not been submitted concurrently elsewhere nor is likely to be published elsewhere in substantially the same form. In the case of papers reviewing a major research programme, the author must certify that the material has not been published substantially in the same form elsewhere and that permission for the Society to publish has been granted by all copyright holders. Letters to the Editor, Discourses, Short Notes and Abstracts of Australian PhD theses may also be submitted for publication. Please contact the Editor if you would like to discuss a possible article for inclusion in the Journal.

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